Somatostatin-14 and somatostatin-28 induce opposite effects on potassium currents in rat neocortical neurons

(prosomatostatin/potassium channels/adenylate cyclase/cerebral cortex)

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ABSTRACT The prosomatostatin-derived peptides somatostatin-14 (Som-14) and somatostatin-28 (Som-28) are believed to act as neurotransmitters in the central nervous system. To examine possible mechanisms by which these peptides induce their physiological actions in brain, the effects of Som-14 and Som-28 on voltage-dependent K+ currents in rat cerebral cortical neurons in culture were examined by using whole-cell patch-clamp techniques. Som-14 increased a delayed rectifier K+ current (IK) in the cortical neurons, while Som-28 reduced IK in the neurons, both in a concentration-dependent manner. Som-14 and Som-28 could induce opposite changes in IK in the same neurons. Elevating intracellular cAMP in the cortical neurons did not modify the effects of Som-14 or Som-28 on IK, indicating that the peptides can regulate this ionic current through cAMP-independent mechanisms. Pretreatment of the neocortical cells with pertussis toxin, which inactivates inhibitory GTP-binding proteins, abolished both Som-14 and Som-28 modulation of IK, indicating that Som-14 and Som-28 receptors are coupled to IK via GTP-binding proteins. These studies show that Som-14 and Som-28 can induce opposite biological effects, suggesting that Som-14 and Som-28, acting through distinct receptors, may function as different neurotransmitters or neuromodulators.

Immunocytochemical studies have shown that three prosomatostatin-derived peptides, somatostatin-28 (Som-28), somatostatin-14 (Som-14), and Som-28-(1–14), are present in the central nervous system (1, 2). Som-28 is a 28-residue precursor for the 14-residue carboxyl-terminal Som-14 and the amino-terminal Som-28-(1–14) (3). A number of studies have shown that Som-14 and Som-28 can function as neurotransmitters or neuromodulators in the nervous system (4, 5). Som-14 and Som-28 can be released from neurons in a calcium-dependent manner (6). Receptors for Som-14 and Som-28 have been identified in the brain (7). Furthermore, these peptides regulate transmitter release in brain and ionic conductances in neurons (4, 8–12).

It is generally believed that Som-28 and Som-14 exert similar biological effects, and both Som-28 and Som-14 have been proposed to interact with the same receptors in the anterior pituitary and central nervous system (5). However, several studies have suggested that Som-28 may act through a receptor population distinct from the one that mediates Som-14 effects. Mandarino et al. (13) and Brown et al. (14) reported that Som-28 is more potent than Som-14 in blocking insulin release from beta cells of the pancreas. In contrast, Som-14 is more potent in inhibiting glucagon secretion from islet alpha cells. Som-28 and Som-14 have been shown to have a reverse rank order of potency in blocking hormone release and calcium mobilization in several clonal cell lines derived from the anterior pituitary (15). Furthermore, separate binding sites for radioactive Som-14 and Som-28 ligands have been suggested to exist in the rat brain (16). Thus, it is possible that Som-28 can bind to specific Som-28 receptors distinct from those responsive to Som-14.

If Som-14 and Som-28 act upon different receptors, they may induce different physiological effects. Such a finding would strongly support the hypothesis that these two peptides are distinct neurotransmitters or neuromodulators. To determine whether Som-14 and Som-28 induce different physiological actions, we have examined the effects of these two peptides on ionic currents in cultured rat neocortical neurons. These cells express somatostatin receptors and the neurons release both Som-14-like and Som-28-like immunoreactivities (17). We report that Som-14 enhances a voltage-dependent potassium current in these cortical neurons, whereas Som-28 decreases the same current. This study shows that Som-14 and Som-28 can induce different biological actions and suggests that they may be distinct neurotransmitters or neuromodulators.

METHODS

Cell Culture. Primary rat cerebral cortical cultures were prepared as previously described (18). Cerebral cortices were removed from 16- or 17-day-old fetuses and dissociated in 0.03% trypsin in Dulbecco’s modified Eagle’s medium (DMEM). Cells were subsequently dispersed by trituration and plated onto 35-mm Petri dishes containing polylysine-coated coverslips at a density of 0.5–1.0 x 10⁶ viable cells per dish in 1.5 ml of growth medium (DMEM supplemented with 10% Hyclone calf serum, 10% Ham’s F-12 medium with 2 mM glutamine, penicillin at 25 units/ml, and streptomycin at 25 µg/ml). Cultures were maintained at 36–37°C in a humidified 5% CO₂ incubator. Proliferation of glial cells was prevented by the addition of cytosine arabinosoribonucleoside to the cultures 7–10 days after the initial subculturing. Rat neocortical neurons cultured for 2–5 weeks were used in the present study.

Electrophysiological Recordings. Neocortical neurons with soma diameters less than 25 µm were voltage-clamped, using the whole-cell version of patch-clamp techniques (19). A Dagan 8900 patch clamp amplifier and a PDP 11/73 computer (Indec systems) were used to apply voltage steps and record induced currents. Resulting potassium currents were filtered through an eight-pole Bessel filter (corner frequency 2.0 kHz) and digitized at 10 kHz with 12-bit resolution, and data were stored on hard disk for off-line analysis.

External solutions used during recording contained 145 mM NaCl, 3 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 10 mM Hepes, and 8 mM glucose, maintained at a pH of 7.3 with

Abbreviations: Som-28, somatostatin-28; Som-14, somatostatin-14(1–14); Som-28-(15–28); Som-28-(1–14), somatostatin-28(1–14); IBMX, isobutylmethylxanthine.

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NaOH. To isolate potassium currents, 5 μM tetrodotoxin and 5 mM CoCl₂ were used to block sodium and calcium currents. Patch electrodes were filled with a solution containing 140 mM KCl, 1 mM MgCl₂, 10 mM EGTA, 10 mM Hepes, pH 7.3 with KOH. Patch pipettes were fabricated from N51A boro-silicate glass and had a resistance of 2–5 MΩ in control saline solutions. Somatostatin analogs and control saline solutions were applied to neurons by using pressure ejection from blunt micropipettes. Recordings were made at room temperature (21–24°C).

RESULTS
Depolarizing voltage steps from a holding potential of −80 mV evoked outward potassium currents in the neurons under conditions in which voltage-dependent sodium and calcium currents were blocked (Fig. 1). Some neurons express both a transient outward K⁺ current (IA) and a delayed rectifier K⁺ current (IK) (Fig. 1A), while other neurons express only IK (Fig. 1C) (20). Since calcium currents were blocked and the electrode contained 10 mM EGTA, calcium-activated K⁺ currents were not a component of the K⁺ currents measured in the present studies. Application of Som-14 onto the cortical neurons induced a concentration-dependent increase of a persistent K⁺ outward current (Figs. 1 and 2). The effects of Som-14 were generally reversible with brief buffer washes. In cells that expressed both IA and IK, Som-14 increased the outward current throughout the duration of the voltage steps in a pattern which suggests a predominant effect on IK (Fig. 1A and B); in cells that expressed only IK, Som-14 facilitated this K⁺ current (Fig. 1C and D). These findings indicate that Som-14

![Diagram A](image1.png)

![Diagram B](image2.png)

![Diagram C](image3.png)

![Diagram D](image4.png)

**Fig. 1.** Effect of Som-14 perfusion on voltage-dependent potassium currents of rat neocortical neurons. Whole-cell recordings of potassium currents were obtained in response to a 140-ms depolarizing pulse to various test voltages (−70 to +40 mV) from a holding potential (VH) of −80 mV. Linear leak and capacitative currents have been subtracted. Current samples at 130 ms of voltage step were used for I–V curves. (A) Potassium currents were recorded from a neuron with two components, an early transient peak, which decays, and a persistent current, which does not decay during the voltage step. These likely represent IA and IK potassium currents, which have been previously described in these cells (20). After the perfusion of 100 nM Som-14 onto this neuron, both peak and plateau potassium currents were enhanced. HBS, Hanks' balanced salt solution. (B) I–V curves for the late components of potassium currents were obtained from the same neuron used in A. The curve plotted with † was obtained via point-by-point subtraction of the control and Som-14 curves and represents the net outward current induced by Som-14. (C) Potassium currents were recorded from a neuron that has only a late component (IK) of potassium current. (D) The I–V curve plotted with † shows that Som-14-induced outward current in the neuron from C.
predominantly enhances $I_K$. Of the 78 cells studied, 53 responded to Som-14 perfusion with an increase in persistent $K^+$ outward current, while 24 showed no response and 1 showed a decrease in potassium currents.

In contrast to the effects of Som-14, Som-28 decreased $I_K$ in the cortical neurons (Fig. 3). Forty of 60 neurons examined showed a decrease in the delayed rectifier $K^+$ currents after Som-28 application. Nineteen cells examined had no response, and one cell responded with an increase in potassium currents. The inhibitory effect of Som-28 on $I_K$ was reversible (Fig. 3 A and C) and concentration dependent (Fig. 2).

To determine whether Som-14 and Som-28 receptors in the same neurons regulate $I_K$, the two peptides were applied in random order to the same cell and the subsequent effect on the outward $K^+$ current was measured (Fig. 4). Of 18 cells examined, 5 responded to Som-28 with a decreased $I_K$ and to Som-14 with an increased $I_K$ (Fig. 4A); 5 cells responded only to Som-28 (decreased $I_K$) and not to Som-14 (Fig. 4B), and 5 cells responded to Som-14 application with an increase in potassium currents but did not respond to Som-28 application (Fig. 4C); three neurons did not respond to either form of somatostatin. These findings suggest that while most neurons...
examined responded to either Som-14 or Som-28, a subpopulation of cells could be affected by both peptides.

Som-28-(1-14)-like immunoreactivity has been reported in the cerebral cortex (2), but Som-28-(1-14) is generally reported to be biologically inactive (5). To determine whether this peptide is biologically active in this system, the effect of 100 nM Som-28-(1-14) on K⁺ currents in the neocortical neurons was examined. In 11 of 12 cells tested, 100 nM Som-28-(1-14) did not affect IK. Those neurons that were unresponsive to Som-28-(1-14) did respond to either Som-14 or Som-28 with changes in IK.

Previously, Som-14 was reported to enhance a K⁺ current that is regulated by muscarinic agonists (M current) in hippocampal neurons (9). This effect of Som-14 could be blocked by the muscarinic agonist carbachol (9). In the presence of 50 μM carbachol, Som-14 (100 nM) still increased the voltage-dependent K⁺ currents in the cultured cortical neurons to an extent similar to that in control cells. This finding suggests that Som-14 effects on IK in neocortical neurons were unrelated to the previously described M current.

Intracellular second messengers such as cAMP have been shown to mediate the regulatory effects of neurotransmitters on voltage-dependent ion channels (21-23). Som-14 is known to decrease cAMP in the brain and peripheral tissues (24). To determine whether the different effects of Som-14 and Som-28 on K⁺ currents in rat neocortical neurons are related to contrasting actions of the peptides on cAMP production, the peptides were tested for their ability to regulate adenylate cyclase activity in membranes of the cultured rat neocortical cells. Som-14 and Som-28 inhibited basal adenylate cyclase activity of rat neocortical cells by 38% ± 3% and 24% ± 2%, respectively. To test the role of cAMP in mediating the effects of Som-28 and Som-14 on IK, 100 μM cAMP and 0.5 mM isobutylmethylxanthine (IBMX, a phosphodiesterase inhibitor) were added to the recording electrode, thereby allowing the intracellular content of cAMP to be greatly elevated, as previously described (25). Under these conditions Som-14 was still able to increase IK and Som-28 decreased this K⁺ current. These findings suggest that the contrasting effects of Som-14 and Som-28 on K⁺ currents are not due to a differential action of the peptides on cAMP formation.

GTP-binding proteins couple somatostatin receptors to various cellular effector systems, including the catalytic subunit of adenylate cyclase (5), Ca²⁺ channels (10), and K⁺ channels (11, 12). One approach to establish whether inhibitory GTP-binding proteins couple receptors to effector systems is through the use of pertussis toxin. Pertussis toxin catalyzes ADP-ribosylation of inhibitory GTP-binding proteins, thereby inactivating those proteins and uncoupling receptors from their effector systems. In neocortical cells pretreated with pertussis toxin (300 ng/ml for 24 hr), Som-14 and Som-28 were no longer able to modulate IK, suggesting that GTP-binding proteins couple Som-14 and Som-28 receptors to the K⁺ channels mediating IK in neocortical neurons.

DISCUSSION

Previous studies have suggested that Som-14 and its precursor Som-28 may act upon different receptors to induce their physiological actions. No study has revealed different physiological actions of Som-14 and Som-28. This being the case, it is not clearly established that the two peptides act through functionally distinct receptors.

In the nervous system, a number of studies have shown that Som-14 can regulate neuronal excitability and the permeability of the cell membrane to various ions. Results from patch-clamp studies have shown that Som-14 increases an inward rectifying K⁺ current in neurons of the locus coeruleus and submucous plexus (11, 12) as well as the voltage-dependent M current in hippocampal and solitary tract neurons (4, 9). Our data demonstrate that, in neocortical neurons in culture, Som-14 increases a voltage-dependent delayed rectifier K⁺ current. We know of no other report that Som-14 can regulate IK in mammalian neurons.

In contrast to the facilitation of IK by Som-14, Som-28 decreased this ionic current. The effect of Som-28 appeared to be directly on the neurons examined. To our knowledge,
this is the first evidence that Som-14 and Som-28 induce different biological actions and supports the hypothesis that the peptides can act through different receptors. Moreover, some neurons appear to express both types of receptors.

Both Som-14 and Som-28 appear to regulate $I_K$ through cAMP-independent mechanisms. This is suggested by their abilities to increase or decrease $I_K$ in cells with high intracellular concentrations of cAMP and the phosphodiesterase inhibitor IBMX. A cyclic-AMP independent mechanism was also proposed for the ability of Som-14 to enhance an inward rectifying $K^+$ current in locus coeruleus and submucous plexus neurons (11, 12). The present findings would also suggest that somatostatin receptors are capable of interacting with multiple cellular effector systems in the same neuron. These effector systems appear to include adenyly cyclase, $K^+$ channels, and, in preliminary studies (26), voltage-dependent $Ca^{2+}$ channels.

Somatostatin receptors in brain are coupled to GTP-binding proteins (25). Recent electrophysiological studies have shown that a number of neurotransmitter receptors are linked to ionic conductance channels by coupling to $G_1$ or $G_0$, or other subclasses of GTP-binding protein (for review, see ref. 27). Both the Som-14 and Som-28 receptors are coupled to the $K^+$ channels mediating $I_K$ via inhibitory GTP-binding proteins. This is indicated by the ability of pertussis toxin to abolish both Som-14 and Som-28 modulation of $I_K$ in neocortical neurons. Since Som-14 and Som-28 induce opposite effects on $I_K$, it is likely that different inhibitory GTP-binding proteins couple Som-14 receptors and Som-28 receptors to the $K^+$ channels mediating $I_K$. The GTP-binding proteins that couple the somatostatin receptor subtypes to the $K^+$ channels in neocortical neurons remain to be identified.

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