0026-895X/90/030357-05$02.00/0

MOLECULAR PHARMACOLOGY, 38:357-361

Lack of Cross-Desensitization of Somatostatin-14 and Somatostatin-28 Receptors Coupled to Potassium Channels in Rat Neocortical Neurons

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Received April 5, 1990; Accepted June 18, 1990

SUMMARY

The effects of somatostatin-14 (SOM-14) and somatostatin-28 (SOM-28) on the delayed rectifier K^+ current (I_K) in rat neocortical neurons in culture were measured by using whole-cell patch clamp techniques. SOM-14 stimulated I_K in a reversible manner. Continuous application of SOM-14 to the neocortical neurons led to a gradual desensitization of the SOM-14 response. Many cells became completely desensitized to SOM-14. SOM-28 also modulated I_K in neocortical cells. However, SOM-28 reduced I_K. This response was also reversible. Continuous application of SOM-28 to neocortical neurons led to a desensitization of the SOM-28 inhibition of I_K. Many of the neurons that responded to SOM-28 became completely refractory to the peptide following prolonged SOM-28 pretreatment. While most neocortical neurons responded either to SOM-14 or to SOM-28, a population of neurons responded to both peptides. Chronic application of SOM-14 to these neurons completely desensitized the SOM-14 stimulation of I_K but did not affect SOM-28 inhibition of this potassium current. Similarly, complete desensitization of SOM-28 responses in these cells was not associated with a modification of SOM-14 stimulation of I_K. The lack of cross-desensitization between SOM-14 and SOM-28 induced responses suggests that these peptides act through different receptors to regulate I_K.

SOM-14 is a neurotransmitter and/or a neuromodulator in the central nervous system (1). This peptide has been shown to regulate neuronal firing activity as well as the release of neurotransmitters and may have a role in centrally mediated behaviors such as movement and cognition (1–6). SOM-14 induces its physiological actions by stimulating membrane-bound receptors, and these receptors in brain have been extensively characterized using a variety of biochemical techniques (1, 7–9). The cellular mechanisms of action of SOM-14 have also been examined using electrophysiological approaches. SOM-14 has been shown to reduce voltage-sensitive Ca^{2+} currents in both peripheral and central neurons (10, 11). Furthermore, SOM-14 stimulates K^+ currents in neurons. In particular, SOM-14 has been reported to increase an inward-rectifying K^+ current in locus coerleus cells (12) and a muscarinic-regulated K^+ current in the hippocampus (3, 6). The stimulation of K^+ currents by SOM-14 may be the underlying event associated with the ability of SOM-14 to hyperpolarize neurons and reduce action potential generation in brain cells (3, 5, 6, 12).

This work was supported by National Institute of Mental Health grant MH 45533, The Juvenile Diabetes Foundation and the Office of Naval Research (N00014-88-K-0048) to T.R., and National Institutes of Health grant NS24927 and a grant from the Pew Foundation Program in Neuroscience to M.D.

Recently, we reported that SOM-14 stimulated a delayed-rectifier K^+ current (I_K) in neocortical neurons (13). This effect was reversible, occurred at low nanomolar concentrations of the peptide, and was selective. Interestingly, SOM-28, another biologically active cleavage product of preprosomatostatin (1), inhibited I_K in neocortical neurons (13). This effect was also reversible, potent, and selective. Most neocortical neurons responded to either SOM-14 or SOM-28 with opposing effects. This finding suggests that SOM-14 and SOM-28 act via different receptors to regulate I_K. Some neocortical neurons responded to both peptides, and in those cells, SOM-14 stimulated and SOM-28 inhibited I_K, further suggesting that the peptides act via different receptors to regulate this K^+ current. These findings indicate that SOM-14 and SOM-28 can induce different physiological actions. This is the first evidence that SOM-14 and SOM-28 may act as distinct neurotransmitters or neuromodulators.

To determine further whether distinct subclasses of receptors mediate the effects of SOM-14 and SOM-28 on I_K, we have tested whether the SOM-14 and SOM-28 responses in neocortical cells can be differentially regulated. Previous studies have shown that somatostatin receptors in pituitary cells desensitize

ABBREVIATIONS: SOM-14, somatostatin-14; SOM-28, somatostatin-28; I_K delayed rectifier K^+ current; DMEM, Dulbecco modified Eagle medium; HBS, Hanks balanced salt solution; TTX, tetrodotoxin; GTP, guanine triphosphate; HEPES, 4-(2-hydroxyethyl)-1-peperazineethanesulfonic acid.

in response to continuous agonist exposure (7, 14–17). In the present study, we show that the modulation of I_K in central neurons by both SOM-14 and SOM-28 rapidly desensitizes and that there is no cross-desensitization between these two peptides. These findings support our hypothesis that distinct SOM-14 and SOM-28 receptors are expressed in brain neurons.

Experimental Procedures

Cell culture. Primary rat cerebral cortical cultures were prepared as previously described (13). Briefly, cerebral cortices were removed from 16- or 17-day-old fetuses, dissociated in 0.03% trypsin-DMEM for 40 min at 37°, and then reincubated for 20 min in HBS (see below). Cells were subsequently dispersed by trituration and plated onto 35-mm petri dishes containing polylysine-coated coverslips at a density of 0.5×10^6 to 10^6 viable cells per dish in 1.5 ml of growth medium (DMEM supplemented with 10% Hyclone calf serum, 10% Ham's F-12 with glutamine, 50 units of penicillin per ml, 25 μg of streptomycin per ml). Cultures were maintained at 36–37° in a humidified 5% CO_2 incubator. Proliferation of glial cells was prevented by the addition of cytosine arabinoside to the cultures 7–10 days after the initial culturing. Rat neocortical neurons cultured for 2 to 5 weeks were used in the present study.

Electrophysiological recording. Neocortical neurons with soma diameters less than 25 μm were voltage clamped by using the whole-cell version of patch clamp techniques (18). 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 2.5 kHz with 12-bit resolution, and the data was stored on hard disk for off-line analysis. Leak currents were averaged, and the leak and capacitative transients were subtracted from all records.

External solutions used during recording contained (mM) 145 NaCl, 3 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 8 glucose maintained at pH 7.3 with NaOH. In order to isolate potassium currents, 5 μM TTX and 5 mM $CoCl_2$ were used to block sodium and calcium currents. Patch electrodes were filled with a solution containing (mM) 140 KCl, 1 $MgCl_2$, 10 EGTA, and 10 HEPES maintained at pH 7.3 with KOH. Patch pipettes were fabricated from N51A borosilicate glass and had a resistance of 2–5 Mohms in control saline solutions. Somatostatin analogs (purchased from Bachem, Torrance, CA and Peninsula, San Carlos, CA), and control saline solutions were applied to neurons by using pressure ejection from blunt micropipettes. Recordings were performed at room temperature (21–24°). Student's unpaired *t* test was used to determine whether the difference between values was significant. Values are presented as the mean ± standard error of multiple determinations.

Results

Recently, we reported that SOM-14 stimulates and SOM-28 inhibits I_K in neocortical neurons in culture (13). As shown in Fig. 1, the responses of neocortical cells to the somatostatin peptides are heterogeneous. Of the 103 neurons tested, 53 responded to 100 nM SOM-14, with an average increase in I_K of 31 ± 2%, and 52 neurons responded to SOM-28, with an average decrease in I_K of 25 ± 1%. Twenty-one neurons responded to both peptides. In these cells, the increase in I_K induced by SOM-14 was 30% ± 2% and the decrease in I_K in response to SOM-28 was 24 ± 1%.

To test whether SOM-14 stimulation of I_K causes desensitization, 100 nM SOM-14 was continuously applied onto 55 neocortical neurons. Twenty-eight cells responded to SOM-14 with an immediate stimulation of I_K (Fig. 2). The other cells tested did not respond to SOM-14. Continuous application of 100 nM SOM-14 desensitized 18 of the cells that responded to SOM-14. Of the cells that desensitized to SOM-14, 10 cells became totally refractory to SOM-14 within 5 min of continuous application of the peptide. The time course for SOM-14 desensitization in these neurons is presented in Fig. 2. Ten cells that responded to SOM-14 did not become refractory to SOM-14 despite the continuous application of the peptide for 5 min. Furthermore, eight other cells were only partially desensitized to SOM-14 after 5 min of peptide treatment of the cells. These cells may have required longer periods of exposure to SOM-14 in order to become desensitized. To test this hypothesis, 18 neocortical cells were treated for 30 min with 500 nM SOM-14, and after this treatment, no cell responded to SOM-14 with an increase in I_K above basal levels (not shown).

SOM-28 inhibition of I_K also desensitized following continuous application of SOM-28 to the cells (Fig. 3). A total of 58 cells were studied, and 27 responded to SOM-28 with a decrease in I_K. The other 31 cells did not respond to SOM-28. Continuous application of SOM-28 desensitized 18 neocortical neurons, with 10 cells becoming completely refractory to the peptide within 5 min of sustained application of the peptide. A time course for the complete desensitization of these cells to SOM-28 is presented in Fig. 3. Nine cells that responded to SOM-28 did not desensitize following 5 min of continuous application of the peptide. Furthermore, eight cells only partially desensitized to SOM-28 following 5 min of exposure of the cells to the peptide. To determine whether longer periods of SOM-28 treatment would completely desensitize all SOM-28-sensitive neocortical cells, 26 cells were pretreated for 30 min with 500 nM SOM-28. After the 30-min pretreatment, none of these cells responded to SOM-28 with changes in I_K (not shown).

To test whether cross-desensitization of SOM-14 and SOM-28 responses occurred in neocortical cells, neurons that responded to both peptides were tested (Fig. 4). In eight cells that completely desensitized to SOM-14, SOM-28 decreased I_K (22 ± 1%) to a similar extent as in controls (Fig. 4). Furthermore, in eight cells that completely desensitized to SOM-28, SOM-14 increased I_K (33 ± 5%) to a similar extent as in controls (Fig. 4). These findings indicate that SOM-14 and SOM-28 responses do not cross-desensitize in neocortical neurons.

Discussion

Most studies have reported that SOM-14 and SOM-28 act upon the same receptors to induce identical physiological effects (1). Recently, however, we reported that SOM-14 and SOM-28 induce opposite effects on I_K in neocortical neurons (13). The contrasting effects of the peptides on the same ionic current suggests, but does not prove, that they act through different receptor subtypes to induce their physiological effects.

To further test whether SOM-14 and SOM-28 act through different receptors to regulate I_K, we determined whether neuronal responses to both peptides can be differentially regulated. Continuous application of SOM-14 desensitized the SOM-14 receptors expressed in most neocortical cells. Furthermore, continuous application of SOM-28 to the neocortical neurons desensitized the SOM-28 responses in many of these cells. In neurons that responded to both peptides, no cross-desensitization to SOM-14 and SOM-28 was observed. This finding supports our hypothesis that SOM-14 and SOM-28 act via different receptors to regulate I_K.

The desensitization of neocortical cells to SOM-14 or SOM-

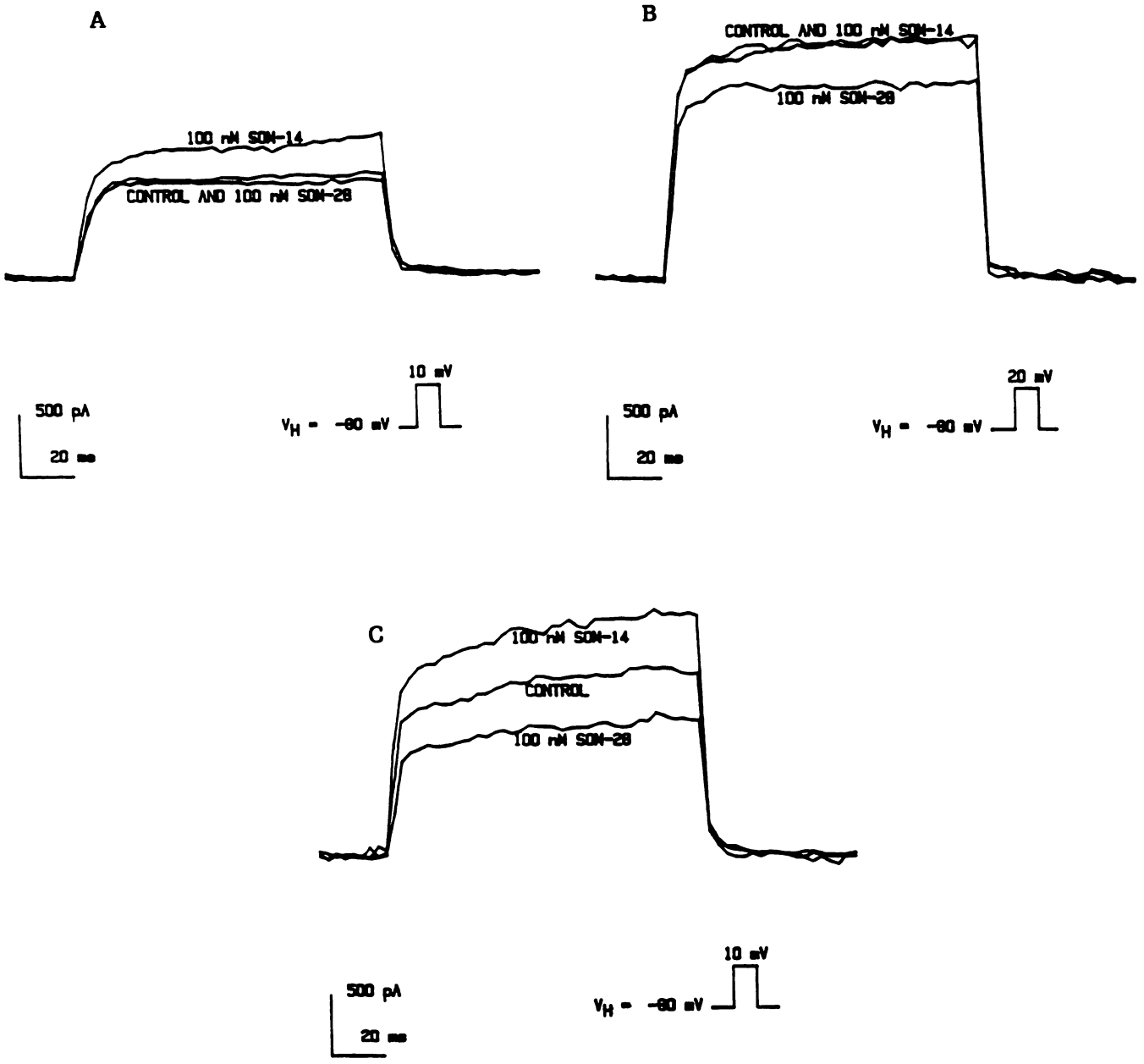

Fig. 1. SOM-14 and SOM-28 differentially regulate I_K in rat neocortical neurons in culture. Whole-cell recordings of K^+ currents were obtained in response to a 120-msec depolarizing pulse from a holding potential (V_H) of −80 mV to the test voltages indicated. Linear leak and capacitance currents have been subtracted. (A) Representative example of a neuron which responded to 100 nM SOM-14 with an increase in I_K but did not respond to a subsequent application of 100 nM SOM-28. Similar results were obtained whether SOM-14 was applied before or after SOM-28 (B) Representative example of a neuron which responded to 100 nM SOM-28 with a decrease in I_K but did not respond to 100 nM SOM-14. Similar results were obtained regardless of the order of the peptide application. (C) Representative example of a neuron which responded to SOM-14 with an increase in I_K and then responded to SOM-28 with a decrease in I_K. Similar results were obtained regardless of the order of the peptide application.

28 was heterogeneous. Some cells rapidly and completely desensitized to either peptide. Other cells only partially desensitized to the somatostatin peptides or were resistant to desensitization during the short periods of exposure of the cells to the somatostatin peptides. Treatment of the neocortical cells with either SOM-14 or SOM-28 for longer periods completely desensitized the neurons to either SOM-14 or SOM-28, respectively. This finding suggests that all neocortical cells desensitize to the somatostatin peptides, but at different rates. The different time courses of somatostatin desensitization suggest that the molecular mechanisms regulating somatostatin receptors vary within different populations of neocortical cells. Interestingly, the regulation of somatostatin receptors in two homogeneous pituitary tumor cell lines, AtT-20 and GH_3, also varies, since continuous application of either SOM-14 or SOM-28 rapidly desensitizes somatostatin receptors expressed in AtT-20 cells but not GH_3 cells (7, 14–17).

The molecular basis of SOM-14 and SOM-28 desensitization in unknown. Previous studies have shown that SOM-14 and SOM-28 desensitization in AtT-20 cells is associated with a down regulation of somatostatin receptors (15). The very rapid desensitization of SOM-14 and SOM-28 responses in neocortical cells suggests that receptor down regulation is unlikely to account for the lost responsiveness to these peptides, and

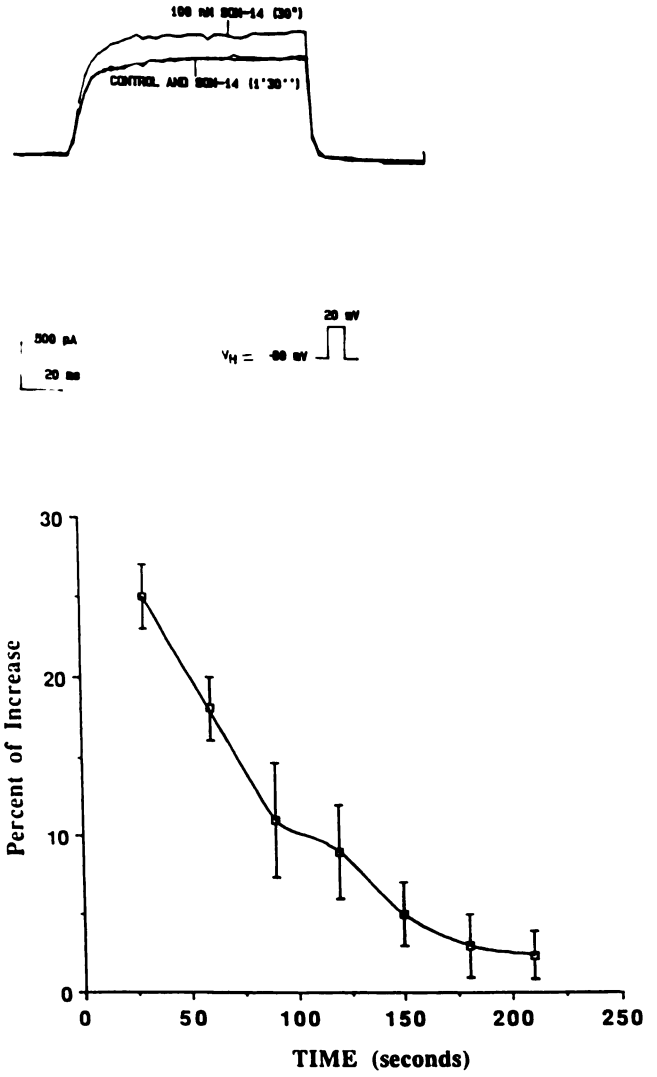

Fig. 2. Desensitization of SOM-14 stimulation of I_K in rat neocortical cells in culture. For these desensitization studies, 100 nM SOM-14 was continuously applied to neocortical cells, and recordings were made at various times after the initiation of the peptide application. At the top are the traces of the recordings from a neuron which completely and rapidly desensitized to SOM-14. At 30 sec after the initiation of the peptide application, a significant stimulation of I_K was observed. However, 90 sec after the start of the peptide application, SOM-14 no longer increased I_K. A time course for the desensitization of the neocortical cells is presented at the bottom. These values are only from cells that completely desensitize to SOM-14 within 5 min of SOM-14 application. The ordinate refers to the percentage increase in I_K induced by 100 nM SOM-14 after SOM-14 treatment for various times. The average initial stimulation of I_K induced by SOM-14 within 30 sec after the initial application of the peptide is 31% ± 2% above basal currents. Each datum point is the mean ± standard error of the percentage change in I_K observed in 10 cells.

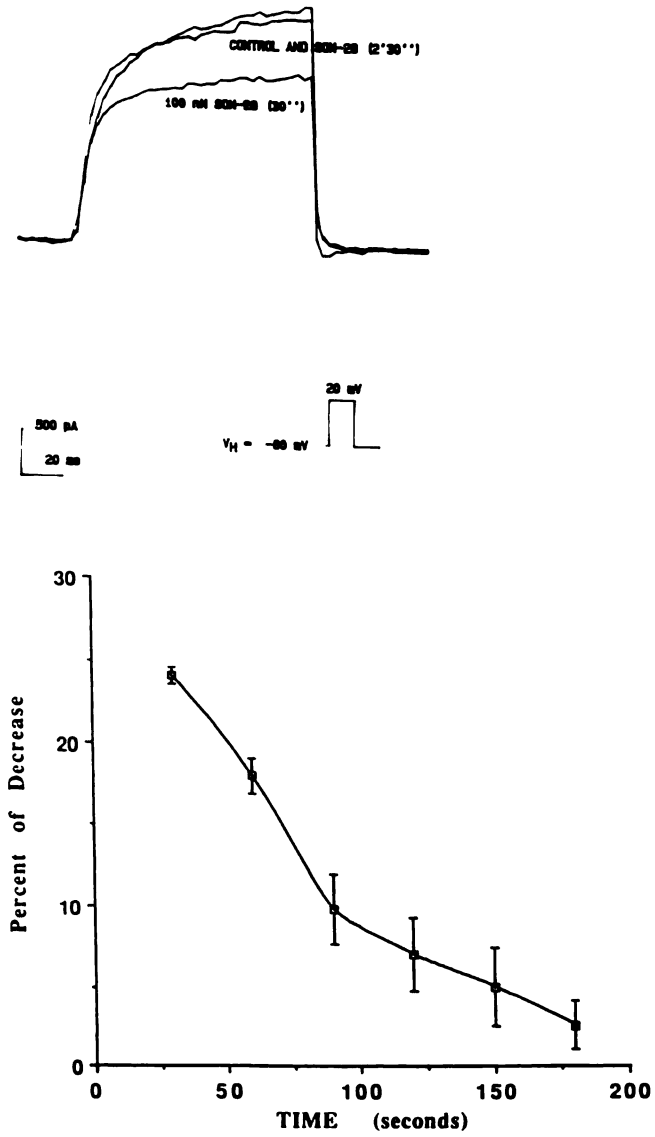

Fig. 3. Desensitization of SOM-28 inhibition of I_K in rat neocortical neurons. For these studies, 100 nM SOM-28 was continuously applied to neocortical neurons, and I_K was measured at various times after the peptide application. A trace of the recordings from a neuron which completely desensitized to SOM-28 is presented at the top. At 30 sec after the application of SOM-28, a significant inhibition of I_K is observed. However, 150 sec after the initiation of the SOM-28 treatment, SOM-28 no longer decreased I_K. A time course for the SOM-28 desensitization is plotted at the bottom for those neurons that completely desensitized to SOM-28. The ordinate represents the percentage decrease in I_K induced by 100 nM SOM-28 as a function of the time of SOM-28 pretreatment. The percentage decrease in I_K induced within 30 sec of SOM-28 application is 25% ± 1% of basal currents. Each point represents the mean ± standard error of 10 different cells.

instead it is possible that the somatostatin receptors become rapidly uncoupled from the G proteins linking the receptors to the K^+ channels. This hypothesis is supported by the finding that the K^+ channels mediating I_K in neocortical cells do not appear to be modified by the somatostatin pretreatments, since basal K^+ current amplitudes were the same as in control and somatostatin-treated cells. Furthermore, in preliminary studies, neocortical cells made refractory to either SOM-14 or SOM-28 respond to internal perfusion of the stable GTP analog, GTP-gamma-thiol, with changes in I_K similar to those in control cells.[1] This finding suggests that during somatostatin desensitization, the ability of endogenous G proteins in neocortical cells to modulate I_K is not altered. Thus, as for other hormone or neurotransmitter receptors, SOM-14 and SOM-28 desensitization may involve a modification of the SOM-14 and SOM-28 receptor subtypes so that they do not easily couple with G proteins, or a physical separation of the somatostatin receptors from G proteins occurs due to receptor internalization

[1] H.-L. Wang, M. Dichter, and T. Reisine, unpublished observations.

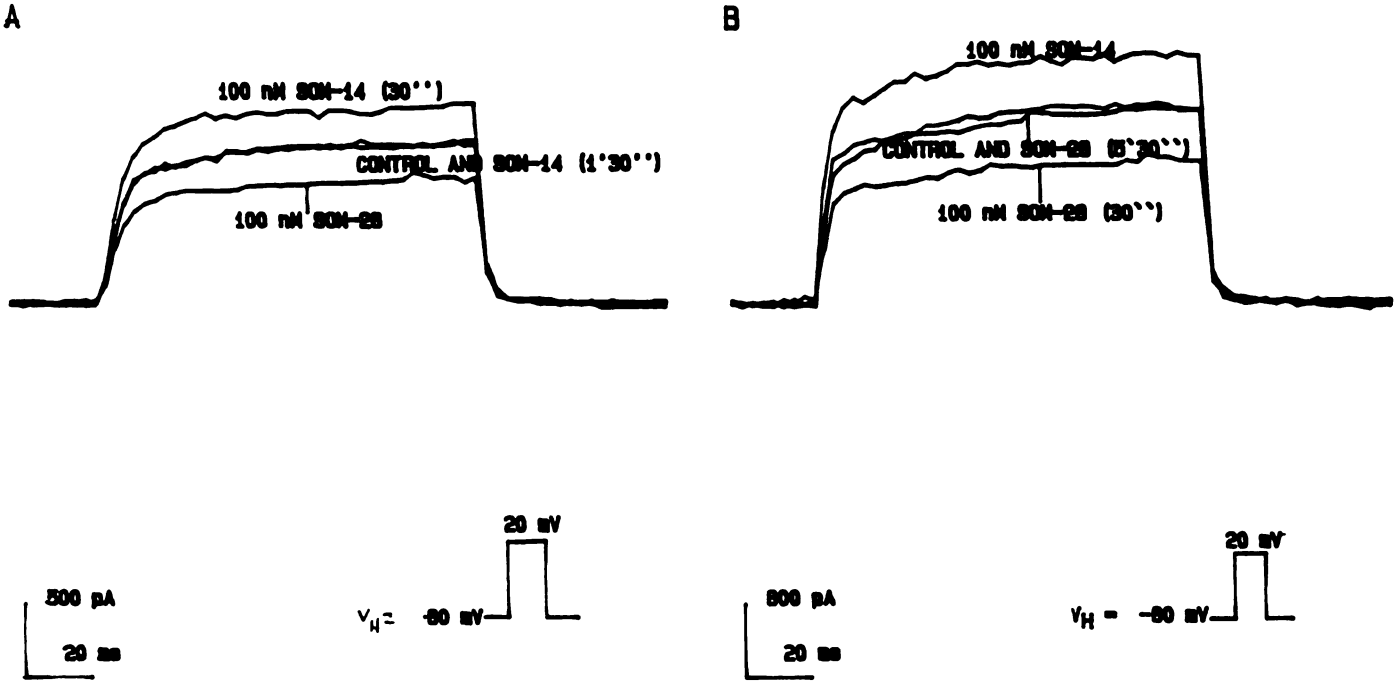

Fig. 4. Lack of cross-desensitization of SOM-14 and SOM-28 responses in rat neocortical cells. To determine whether SOM-14 and SOM-28 modulation of I_K cross-desensitize, neocortical neurons were tested that responded to both peptides. (A) Example of a neocortical neuron which desensitized to SOM-14 but responded to SOM-28 with a decrease in I_K. For this neuron, 100 nM SOM-14 was continuously applied, and at 30 sec after the initial application of SOM-14, I_K was significantly increased. However, 90 sec after the start of the SOM-14 application, I_K was back to basal levels. 100 nM SOM-28 was then applied, and it inhibited I_K. (B) Example of a neocortical neuron which desensitized to SOM-28 but still responded to SOM-14 with an increase in I_K. For these studies, 100 nM SOM-28 was continuously applied to the cell. Thirty seconds after the start of the peptide application, I_K was significantly reduced. However, after 330 sec of SOM-28 application, I_K was similar to basal levels. Under these conditions, application of 100 nM SOM-14 significantly increased I_K.

or sequestration to prevent somatostatin modulation of the K^+ currents in neocortical cells. Interestingly, the lack of cross-desensitization of SOM-14 and SOM-28 in neocortical neurons in culture that respond to both peptides suggests that SOM-14 and SOM-28 receptor subpopulations are independently regulated in these cells.

Besides SOM-14 and SOM-28, no other pharmacological agents are available to distinguish the somatostatin receptor subclasses in neocortical cells. For this reason, these desensitization studies were essential to establish clearly that SOM-14 and SOM-28 act through different receptors to induce their physiological actions in cortical neurons. Development of somatostatin receptor subtype selective compounds will be useful for further characterizing the properties of the SOM-14 and SOM-28 receptors and in elucidating the distinct physiological roles of SOM-14 and SOM-28 in the central nervous system.

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