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Somatostatin depresses excitability in neurons of the solitary tract complex through hyperpolarization and augmentation of I_M, a non-inactivating voltage-dependent outward current blocked by muscarinic agonists

(somatomedin-release-inhibitory factor/K^+ conductance)

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ABSTRACT **The synaptic function of somatostatin-containing fibers in the nervous system is controversial. Therefore, we used a slice preparation of the rat brain stem to test the electrophysiological effects of prosomatostatin-derived peptides on neurons of the solitary tract complex, which contains an abundance of somatostatin-containing fibers and cell bodies. Superfusion of both somatostatin-14 and somatostatin-28 (the precursor for somatostatin-14), but not somatostatin-28-(1–12) or -(1–10), predominantly inhibited spontaneous spike and subthreshold (probably synaptic) activity. In intracellular recordings, somatostatin-14 and -28 hyperpolarized most neurons in association with a slight (10–35%) but reproducible decrease in input resistance. These hyperpolarizing responses were augmented in depolarized cells and persisted in cells in which spontaneous inhibitory postsynaptic potentials became depolarizing after Cl^- injection. These data suggest that somatostatin receptors regulate a K^+ conductance. In voltage-clamp studies, somatostatin-28 and -14 induced a steady outward current and augmented the voltage-dependent, nonactivating outward K^+ conductance (I_M) shown to be blocked by activation of muscarinic cholinergic receptors. These results suggest (*i*) that somatostatin-containing elements in the solitary tract complex play an inhibitory role through the activation of postsynaptic permeability to potassium ions and (*ii*) that the same ion channel type may be coregulated by two neurotransmitter candidates, somatostatin and acetylcholine, through a reciprocal control mechanism.**

The solitary tract complex (STC), including the nuclei of the tractus solitarius and the dorsal vagal motor nucleus, is a dorsomedial brain stem structure regulating visceral functions. Excitatory and inhibitory synaptic activity occurs in the STC (1–3). To date, the available information on inhibitory synaptic potentials seen in the medulla oblongata indicates that they are generated by large, rapid increases of membrane conductance, mainly to Cl^-, that can be mimicked by application of glycine and γ-aminobutyric acid (2, 3). In respiratory neurons *in vivo*, this Cl^- mechanism mediates potent and fast suppression of neuronal activities following an afferent impulse or during certain phases of the respiratory cycle (4, 5).

Another potential inhibitory agonist found in the STC (6, 7) is the tetradecapeptide somatostatin [somatostatin-14 (SS14) or somatomedin-release-inhibitory factor]. Despite considerable physiological research and abundant immunohistochemical data showing wide-spread somatostatin-containing cell bodies and fibers in several brain areas, there is disagreement on the primary effects of these somatostatin (SS) elements (see ref. 8 for review). Administration of SS has been reported to inhibit (see, e.g., refs. 8–15) and to excite (8, 16–19) the discharge of central neurons *in vivo* and *in vitro*. In intracellular recordings from hippocampal slices *in vitro*, depolarization with enhanced spiking (18, 19) and hyperpolarization with depressed spiking (11, 12, 14, 15) have been recorded from the same cell type (CA1 pyramidal cells) after SS administration. The hyperpolarizations are thought to be produced by an increase in K^+ conductance (11, 15). It has been suggested that the SS-evoked excitations observed might derive from enhancement by SS of acetylcholine-induced excitatory effects (13).

A further complication is the suggestion that several different fragments of prosomatostatin-derived peptides exist in nerve elements in, among other brain regions, the hippocampus (20) and the STC (ref. 7 and L. Koda, R. Benoit, N. Ling, C. Bakhit, S.M., and G.R.S., unpublished data). We have reported data that two of these fragments, SS14 and SS28, have potent inhibitory effects on neurons of the STC (21). The present study (reported in abstract form, ref. 22) demonstrates the hyperpolarization and transmembrane currents underlying these inhibitory effects and describes one probable mechanism: an augmentation by SS14 and SS28 (but not other prosomatostatin-derived peptides) of a voltage-dependent conductance probably to potassium ions (the non-inactivating voltage-dependent outward current blocked by muscarinic agonists, I_M).

METHODS

Slices of rat brain stem were prepared as reported (1–3, 23, 24). Briefly, male Wistar or Sprague–Dawley rats weighing 100–170 g were briefly anesthetized with halothane or ether, craniotomized, and decapitated at the upper cervical spinal cord. After transcollicular section, the brain stem was removed, immersed in cold (10°C) artificial cerebrospinal fluid (ACSF) and separated from the cerebellum. During these surgical procedures, cold (10°C) ACSF was dripped onto exposed brain surfaces. (ACSF = 124 mM NaCl/5 mM KCl/2 mM $MgSO_4 \cdot 7H_2O$/1.25 mM KH_2PO_4/26 mM $NaHCO_3$/2 mM $CaCl_2$/10 mM glucose.) The ACSF was gassed with 95% O_2/5% CO_2 in all cases. Coronal slices (350–500 μm thick) were cut on a tissue chopper, placed in cold ACSF, and transferred to warm (31–37°C) ACSF in the

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Abbreviations: STC, solitary tract complex; *I–V*, current–voltage; I_M, non-inactivating voltage-dependent outward current blocked by muscarinic agonists; I_A, transient outward current; SS14, somatostatin-14; SS28, somatostatin-28; SS, somatostatin; ACSF, artificial cerebrospinal fluid.

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recording chamber. The upper surfaces of the slices were first exposed to warm humidified 95% O_2/5% CO_2 for 10–45 min, whereupon the slices were completely immersed and continuously superfused at a constant rate (1–5 ml/min).

Solutions of the SS analogues (obtained from Jean Rivier and Nick Ling at the Salk Institute) were made up immediately before use in ACSF gassed with 95% O_2/5% CO_2 from frozen stock solutions (1 mM). Glass micropipettes containing 3 M KCl or 2 M KOAc of 50–80 MΩ (KCl) or 80–150 MΩ (KOAc) tip resistance, coupled to a Dagan 8100 (Minneapolis, MN) or Axon Instruments (Burlingame, CA) Axoclamp amplifier, were used to perform current-clamp and voltage-clamp recording. Single-electrode discontinuous voltage-clamp of the neurons was performed as described (23), using KCl pipettes and the high-frequency switching, current-injection and voltage-sampling method. The sampling frequency was set manually to 2.5–5 kHz with a duty cycle of 30 or 50%. Apparent input resistance and reversal or "null potentials" for SS-induced responses were estimated in current-clamp mode by injection through the recording pipette of steps of hyper- and depolarizing current steps, for construction of current–voltage (*I–V*) curves. In voltage-clamp mode, voltage step commands of various amplitudes (0.8- to 1-sec duration) were delivered from various holding potentials, and instantaneous and steady-state *I–V* curves were constructed before, during, and after SS superfusion.

RESULTS

A total of 16 cells were studied by extracellular recording and 31 cells were studied by intracellular recording. The basic electrophysiological properties of these neurons have been reported elsewhere (1, 2, 23). In spontaneously firing STC neurons, SS14 and SS28 superfusion (0.1–2 μM) depressed discharge frequency (Figs. 1 and 2) in the great majority of neurons tested. Thus, in 26 neurons tested with SS14, 10 of 16 recorded intracellularly and 7 of 10 recorded extracellularly, showed this response. Likewise in 15 neurons tested with SS28, 7 of 9 recorded intracellularly and 5 of 6 recorded extracellularly, showed this response. The depressions were characteristically slow in onset (even at high superfusion rates) and slow to recover (Fig. 1) after washout with ACSF alone, especially after SS28 superfusion. Thresholds for the inhibitions appeared to be ≈0.1 μM for SS14 and SS28. Superfusion of somatostatin-28-(1–12) also depressed discharge frequency (with little or no change in membrane potential) in some neurons (4 of 7 tested intracellularly; 0 of 2 extracellularly), but only at high concentrations (0.5–5 μM). A control peptide, somatostatin-28-(1–10), excited 1 neuron and had no effect on 7 other neurons (n = 5 intracellularly and n = 2 extracellularly recorded) at 0.5–5 μM concentrations. Excitatory responses were seen with SS14 superfusion in only 3 of 26 cases, and no excitations were elicited by SS28 or somatostatin-28-(1–12).

In intracellular recordings, neurons of the STC displayed membrane potentials of −50 to −70 mV, near or above the threshold for spontaneous discharge of action potentials (−45 to −55 mV; see refs. 1–3, 23). SS14 hyperpolarized 10 of 16 cells (by 5–20 mV; Figs. 1 and 2), depolarized 1, and had no measurable effect in 5 cells. SS28 hyperpolarized 7 cells and had no effect in 2 cells. The SS-induced hyperpolarizations were smallest in those cells with more negative membrane potentials (−55 to −70 mV). Hyperpolarizations were accompanied by decreases in the frequency of spontaneous subthreshold potentials (presumed postsynaptic potentials). The hyperpolarizations appear to be a direct postsynaptic effect, as they were still elicited during concomitant superfusion of 1 μM tetrodotoxin to isolate the cell from synaptic input (in 1 of 3 cells excited by SS14, tetrodotoxin blocked this response). Neither somatostatin-28-(1–12) nor -(1–10) consistently hyperpolarized STC neurons (at 0.5–5 μM concentrations) in the same cells that showed SS14- or SS28-evoked hyperpolarizations, suggesting that specific SS receptors are involved in the inhibitory responses to SS28 and SS14 (see ref. 25).

The SS-evoked hyperpolarizations persisted in cells in which sufficient Cl^- was ejected from KCl-containing recording pipettes to invert presumed (spontaneous) inhibitory postsynaptic potentials to depolarizations (see refs. 1 and 26). In these cells, the hyperpolarizations elicited by SS28 and SS14 were reduced when the membrane potential was hyperpolarized by 10–20 mV with intracellular current injection; concomitantly, the amplitude of synaptic potentials, including reversed chloride-dependent potentials, was increased by the same procedure. Thus, the SS-induced hyperpolarizations are not likely to involve increases in Cl^- conductance, in contrast to γ-aminobutyric acid type A receptor-activated hyperpolarizations (8, 26). Measurement of the *I–V* relationship during hyperpolarization by the SSs (Fig. 2) showed a decreased input resistance (by 10–35%) and null potentials or extrapolated reversal potentials ranging from −70 to −90 mV. Thus, an increase in K^+ conductance is probably the mechanism underlying the hyperpolarizations and the resulting inhibition of discharge. A decreased input resistance occurred with SS14 (Fig. 2 *d–f* and *h*) in six neurons and with SS28 (Fig. 2 *a–c* and *g*) in four neurons, but not with somatostatin-28-(1–12) or -(1–10).

A possible action of SS28 and SS14 superfusion on a voltage-dependent K^+ current was investigated in current-clamp and voltage-clamp recordings, using those neurons exhibiting a more pronounced decrease of the input resistance during the SS-induced hyperpolarization. We found no SS effect on the afterhyperpolarization (AHP) following single spontaneous action potentials or following repetitive spike discharge induced by depolarizing current injection. Furthermore, the size or duration of tetrodotoxin-resistant action potentials (probable Ca^{2+} spikes) was not modified. Therefore, we tentatively conclude that SSs do not significantly interact

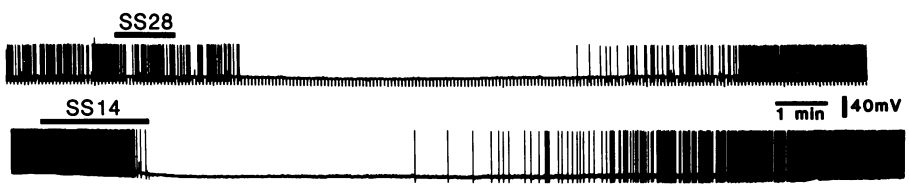

FIG. 1. SS28 (upper trace, 0.5 μM) and SS14 (lower trace, 0.2 μM) induce membrane hyperpolarization and inhibit spontaneous spike discharge. Intracellular voltage recording of the membrane potential from two different STC neurons located in the ventral subnucleus of the solitary tract. Recording electrodes contained 3 M KCl in both cases. SS applications are indicated by bars above tracings. Voltage recordings were played back at slow speed from a magnetic tape recorder to improve reproduction of high-frequency signals by the chart recorder. For the upper trace, initial membrane potential = −55 mV; SS28-induced hyperpolarization = 5 mV; downward voltage deflections were induced by hyperpolarizing current injections of 0.2 nA and were reduced by 15% in amplitude during inhibition by SS28. For the lower trace, initial membrane potential = −40 mV; SS14-induced hyperpolarization = 15 mV. Note that the largest hyperpolarizing effect occurs at the more depolarized membrane potential (lower trace) and that both responses peak at −60 to −65 mV.

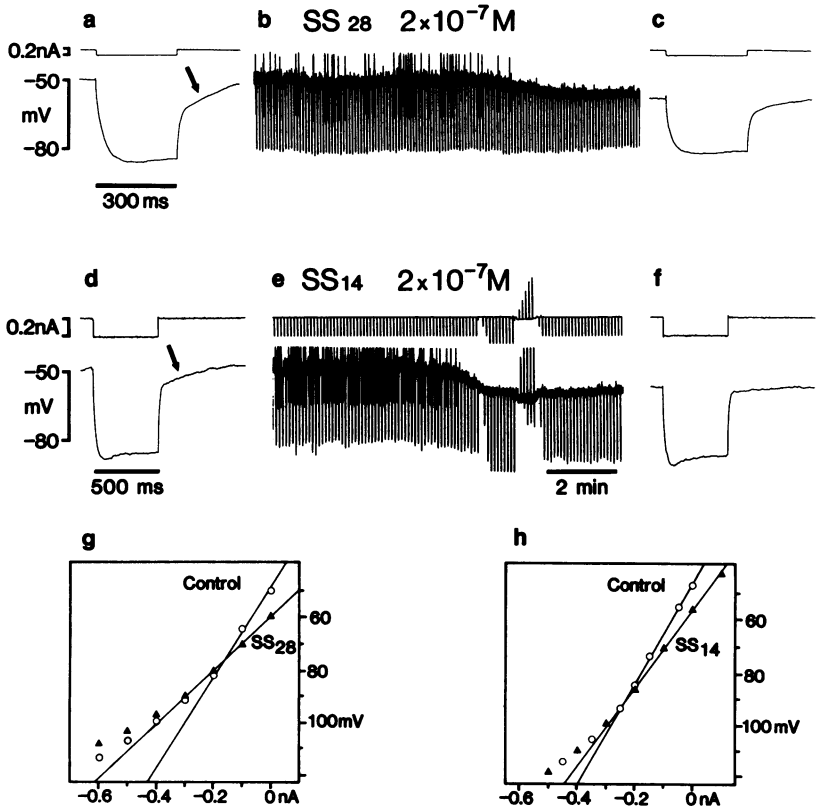

FIG. 2. Input resistance of STC neurons is decreased by SS28 (*a–c* and *g*) and SS14 (*d–f* and *h*); voltage recordings of two different neurons from the ventral subnucleus of the tractus solitarius. Recording pipettes contained 3 M KCl in both cases. Square pulses of −0.2 nA hyperpolarizing current (upper trace in *a*, *c*, *d*, *e*, and *f*) are injected through the recording electrode during extracellular administration of SS. Chart recordings at slow speed (*b* and *e*) and voltage transients at higher speed (*a*, *c*, *d*, and *f*) are shown. The time calibration for *a* applies also to *c*, that for *d* also applies to *f*, and that for *e* also applies to *b*. Voltage transients are digitized averages of five successive samples taken before (to the left in *a* and *d*) and during (to the right in *c* and *f*) the action of SS. Initial membrane potential in both cases was −50 mV. SSs suppressed the firing of action potentials (truncated by the slow polygraph rise-time), hyperpolarized the membrane and decreased the amplitude of the induced voltage transients. (*g* and *h*) *I–V* curves. Reversal potentials were estimated from the intersection of *I–V* curves before and during the action of SS. Reversal potential was extrapolated from measurements taken between −45 and −90 mV membrane potentials. Amplitude of the voltage transient was measured at 300 ms after the onset of the current pulse. (*a–c* and *g*) Decrease of input resistance was 35%; the reversal potential was −75 mV. (*d–f* and *h*) Decrease of input resistance was 17%; the reversal potential was −90 mV. Voltage trajectories (arrows) following offset of the hyperpolarizations suggest activation of I_A in control conditions (*a* and *d*) that appears reduced during the action of SS (*c* and *f*); this is more likely a consequence of SS-induced hyperpolarization to levels near (*a–c*) or below (*d–f*) I_A activation thresholds and of the reduced amplitude of the step (pulse) hyperpolarization rather than a direct effect of SS on I_A (see ref. 23 and voltage-clamp data in Fig. 3).

with Ca^{2+} currents, Ca^{2+}-dependent K^+ currents, or the delayed rectifier current in STC neurons. However, additional, more direct studies will be required to reveal a possible participation of Ca^{2+} currents (see, e.g., refs. 27 and 28). No clear or consistent effect of the SSs on the transient outward current (I_A) was detected (see Fig. 3*A*).

However, we did find evidence for an augmentation by the SSs of a time- and voltage-dependent, noninactivating K^+ current likely to represent I_M (see refs. 23 and 29–31). The neuron shown in Fig. 3 was injected with Cl^- ions and voltage-clamped in the presence of tetrodotoxin. SS28 superfusion caused a reversible, steady outward current with the membrane potential held at −40 mV. In the control situation, hyperpolarizing voltage commands from holding potentials of −40 to −45 mV caused inward current relaxations (i.e., a slow "sag" from the instantaneous to the steady-state condition; see refs. 23 and 29–31) with an extrapolated activation threshold close to −70 mV (Fig. 3 *d* and *e*). The relaxations were associated with a decreased input conductance (i.e., a decreased amplitude of instantaneous or ohmic current jumps at the end compared to the onset of command steps to −45 mV; see Fig. 3*b*) and, therefore, corresponded to the time-dependent suppression of a steady permeability present at −40 mV. In four of five STC cells tested, these relaxations (and the ratio of onset/offset ohmic jumps; Fig. 3*b*) became reversibly larger in the presence of SS28 or SS14 (Fig. 3 *b–e*), whereas their threshold potentials (about −70 mV) were not affected (Fig. 3 *d* and *e*). As reported for muscarine in the STC (23), the relaxations were reduced or abolished in one cell tested by superfusion of the muscarinic agonist carbachol (carbamylcholine chloride) (Fig. 4). The smaller current relaxations evoked by hyperpolarizing commands from a holding potential of −65 mV were not affected by the SSs (three cells), in accord with hippocampal data (32, 33).

Therefore, it is likely that at least a portion of the SS-induced steady outward current elicited at −40 mV holding potentials corresponds to the exaggeration of a steady, voltage-dependent permeability of the neuron probably involving K^+ (the I_M). However, as the ohmic step at the end of the command pulses to −65 mV (when the I_M should be mostly deactivated; refs. 23 and 29–31) was still larger in some STC cells during SS28 than in the control situation, it is possible that the SSs may also open other K^+ channels (e.g., the inward rectifier) in addition to the "M channels" in some STC cells. Additional studies will be required to test this possibility.

DISCUSSION

These results further support claims (see, e.g., refs. 8–15), including data on vagal motor neurons (34), that SS14 has

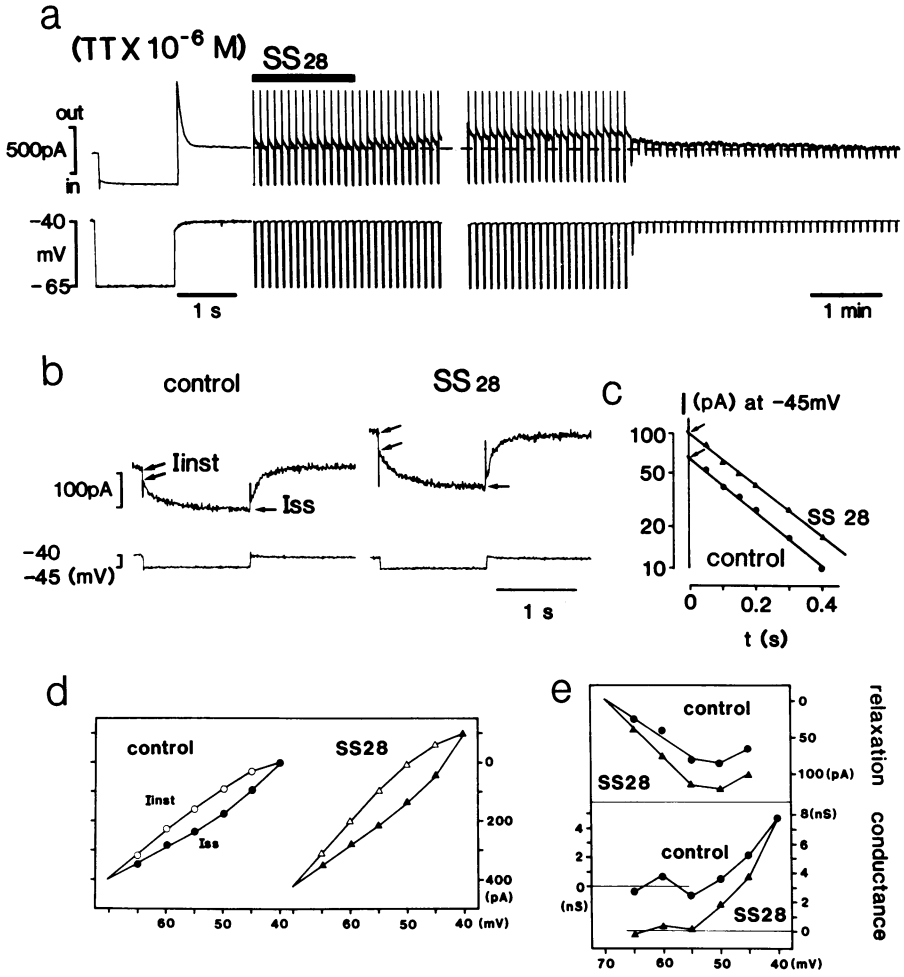

FIG. 3. Voltage-clamp recording of outward currents in a nucleus tractus solitarius neuron tested with 0.5 μM SS28. Holding potential = −40 mV. For the KCl (3 M)-containing electrode, resistance = 70 mΩ. (*a* and *b*) Chart recording and samples at faster speed (five traces digitized and averaged) of current (upper traces) and voltage (lower traces). Note that SS reversibly induced a steady outward current (maximal amplitude = 100 pA) that developed 1–5 min after the onset of SS superfusion and reversed 8 min later (horizontal dotted line indicates control current level). Steps of voltage command to −65 mV (left-hand side of *a*) and changed to −45 mV (right-hand side of *a*). Two minutes of recording, during which a wider range of hyperpolarizing commands was varied for construction of *I–V* plots (see *d*), are omitted for clarity (empty space between left and right traces). Recordings at a faster time base in *a* are taken immediately before SS28 application (voltage command to −65 mV, left hand side). (*b*) The same cell specimen records of current relaxations during hyperpolarizing commands to −45 mV before (control) and during SS28. Note that the instantaneous (ohmic) current step at the beginning of the command (Iinst; double arrows) is larger than that at command offset or steady state (Iss; single arrow), indicating that the inward relaxation corresponds to the time-dependent suppression of a steady outward current (see ref. 29). Note that SS28 increases the current relaxation as well as the initial ohmic step (double arrows) and causes an outward current at the holding potential. Recovery from all these effects was recorded but is not shown (see recovery from the outward holding current effect in *a*). Instantaneous current at the end of the command to −65 mV in *a* cannot be measured because of the activation of a fast transient outward current (probably I_A). The apparent reduction of I_A by SS28 arises as a result of attenuation by the slow rise-time of the polygraph. (*c*, *d*, and *e*) Effect of SS28 on inward relaxations in the same neuron. (*c*) Estimation of the amplitude of relaxations at the −45 mV command potential before (control) and during SS28 action; logarithmic plots of relaxation current vs. time are fitted by single exponential curves, the time constant of which was not significantly changed by SS application. The time constants are equivalent to those reported for the I_M in these neurons (23). The amplitude of relaxations was measured by extrapolating current back to the onset of the hyperpolarizing command (small arrows). (*d*) *I–V* curves constructed from the instantaneous (Iinst onset) and steady-state (Iss) currents, before (control) and during SS28. Instantaneous and steady-state currents are defined as in *b* and measured as in *c*. Note extrapolated intersection of the curves of about −70 mV in both. (*e*) Amplitude of relaxation (determined as described above) and underlying conductance as a function of membrane potential. Change of conductance underlying the relaxation was calculated by dividing the amplitude of relaxation by its driving force (the difference between the membrane potential and the equilibrium potential). This conductance is ≈4 nS in control conditions, and it is nearly doubled by SS28. The conductance present during hyperpolarizing steps from the holding potential (−40 mV) does not decrease further at commands more hyperpolarized than −50 to −55 mV. Conductance is plotted on an ascending scale for clarity.

predominantly inhibitory actions in the central nervous system. The STC is known to contain an abundance of fibers and cell bodies containing various neuropeptides, including SS (6, 7). Indeed, preliminary immunohistochemical studies of the STC (ref. 7; L. Koda, R. Benoit, N. Ling, C. Bakhit, S.M., and G.R.S., unpublished data) have shown cell bodies containing SS28 and a dense network of fibers immunoreactive for somatostatin-28-(1–12) and SS14. From the present results, the role of somatostatin-28-(1–12) would seem to be less important than that of SS28 or SS14. However, our results suggest that both SS28 and SS14 should be considered candidates for inhibitory neurotransmitters in the STC. This brain stem structure is known to be involved in the central control of the autonomic nervous system. Our findings and studies showing that microinjection of SSs into the STC *in vivo* dramatically alters blood pressure (7) and respiration (35) thus point to their role in autonomic control.

Several considerations support an integrative role for the SSs. Compared to the rapid Cl^--dependent inhibitions such as those evoked by γ-aminobutyric acid, SS does not change

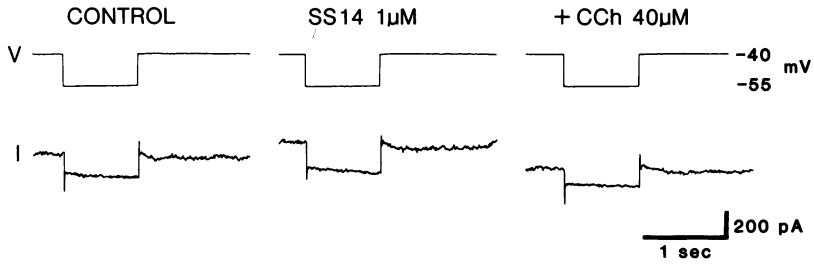

FIG. 4. Effects of SS14 and carbachol (CCh) on current relaxations in another neuron from the nucleus tractus solitarius during voltage-clamp recording. Holding potential = −40 mV, with −15 mV hyperpolarizing commands. Superfusion of 1 μM SS14 increases the inward current relaxation (by 77%) and the instantaneous ohmic step, while causing a steady outward current. Carbachol (40 μM) reverses both of these SS effects and in addition reduces the current relaxation (by 55%) and the instantaneous ohmic step compared to control, while causing a net inward steady current at the holding potential. A small outward tail current at command offset (probably I_A) prevents analysis of the ohmic step from steady state (KCl recording pipette; 3-kHz switching frequency; resting membrane potential was −55 mV).

input resistance markedly; therefore, its shunting action on fast potential transients, such as presumed postsynaptic potentials, is probably weak. However, SS readily hyperpolarizes for long durations those neurons with membrane potentials close to the threshold for spike firing (−45 to −55 mV). This may be a selective mechanism to adjust propagated trains of activity (e.g., intrinsically generated) without significantly affecting the incoming dendritic or synaptic signals. Augmentation of the I_M would also be expected to enhance the stabilizing or potential clamping effect of the I_M at or near resting potentials (31).

There is evidence that SSs, although directly inhibitory, actually can augment the responses of hippocampal neurons to both excitatory (acetylcholine) and inhibitory (γ-aminobutyric acid) transmitters (13, 14). The present results provide an explanation for the augmentation of cholinergic (muscarinic) responses *in vivo* (13). Several lines of evidence suggest that the SSs activate the I_M. (*i*) The current relaxations augmented by the SSs exhibit amplitude, kinetic, and voltage-dependent properties identical to the I_M characterized in these STC neurons (23). (*ii*) Muscarinic agonists block the relaxations in STC neurons (also see ref. 23). (*iii*) Both Ba^{2+} and muscarinic agonists block the SS-induced augmentation of the current relaxations and the hyperpolarizations seen in hippocampus (15, 32, 33). (*iv*) The relaxations are associated with a decreased ohmic (instantaneous) step at the end of hyperpolarizing commands compared to the ohmic step at the beginning, both in STC and hippocampal (23, 32, 33) neurons, and this difference persists during SS perfusion. As far as we are aware, this and the data on hippocampus (32, 33) are the first evidence for a neurotransmitter candidate activating I_M (muscarinic receptor agonists and certain peptides inactivate it; refs. 8, 23, 29–31). Our data also suggest that acetylcholine might be rendered more effective if more M channels were being opened by SS for muscarinic agonists to subsequently close.

Further studies will be required to determine the selectivity of this effect of SS on I_M. Other agonists (e.g., opioids, α-adrenergic agonists, adenosine, and baclofen) are known to activate voltage-dependent (inward rectifier) K^+ conductances in several central nervous system neuron types (36–39) and also might activate I_M. However, the present findings do suggest that at least the SSs, like I_M (29–31), will likely play an important role in suppressing burst discharges and depolarizations much beyond thresholds for spiking, provided acetylcholine is not also present.

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