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Somatostatin Augments the M-Current in Hippocampal Neurons

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Immunocytochemical and electrophysiological evidence suggests that somatostatin may be a transmitter in the hippocampus. To characterize the ionic mechanisms underlying somatostatin effects, voltage-clamp and current-clamp studies on single CA1 pyramidal neurons in the hippocampal slice preparation were performed. Both somatostatin-28 and somatostatin-14 elicited a steady outward current and selectively augmented the noninactivating, voltage-dependent outward potassium current known as the M-current. Since the muscarinic cholinergic agonists carbachol and muscarine antagonized this current, these results suggest a reciprocal regulation of the M-current by somatostatin and acetylcholine.

IMMUNOHISTOCHEMICAL STUDIES indicate that prosomatostatin-derived peptides are present in intrinsic neurons of the hippocampus (*1*). These peptides include somatostatin-28 (SS28) and its cleavage fragments SS28(15–28) (SS14; somatotropin release-inhibiting factor) and SS28(1–12). Other histochemical studies have shown a profuse innervation of the hippocampus by fibers containing markers for acetylcholine (ACh) (*2*) that appear to project to the same dendritic areas of hippocampal neurons as the SS-containing fibers (*1–3*). Electrophysiological studies indicate that activation of cholinergic muscarinic receptors excites pyramidal neurons, probably by means of multiple mechanisms, including presynaptic actions, reduction of Ca^{2+} currents, and suppression of several different K^+ currents (*4–8*).

However, the function of SS14 in the hippocampus is less clear. In some studies, SS14 or SS28 elicited inhibitions and hyperpolarizations of CA1 pyramidal neurons with a reduction of input resistance (*7, 9–11*), interpreted as an increase in K^+ conductance. Excitatory effects of SS14 have also been reported (*12*). These disparate results may derive from methodological differences or from the recently described interaction of ACh and SS: when applied alone, SS elicited only inhibitory effects but enhanced the excitatory effects of ACh on cortical and hippocampal neurons (*13*). Thus, excitatory SS14 effects (*12*) could result from the presence of exogenous or endogenous ACh.

We have pursued the mechanisms of this interaction (*14*) of SS and ACh by using intracellular recording techniques in the rat hippocampal slice, prepared as described (*9, 15, 16*). The slices were completely immersed in a temperature-controlled recording chamber and superfused with standard artificial cerebrospinal fluid (ACSF) to which drugs and peptides were added (*15, 16*). Current- and voltage-clamp (*17–19*) studies were performed with an Axoclamp preamplifier. A total of 39 pyramidal neurons met criteria for lack of penetration injury (*15*). Resting membrane potentials ranged from −54 to −75 mV and averaged

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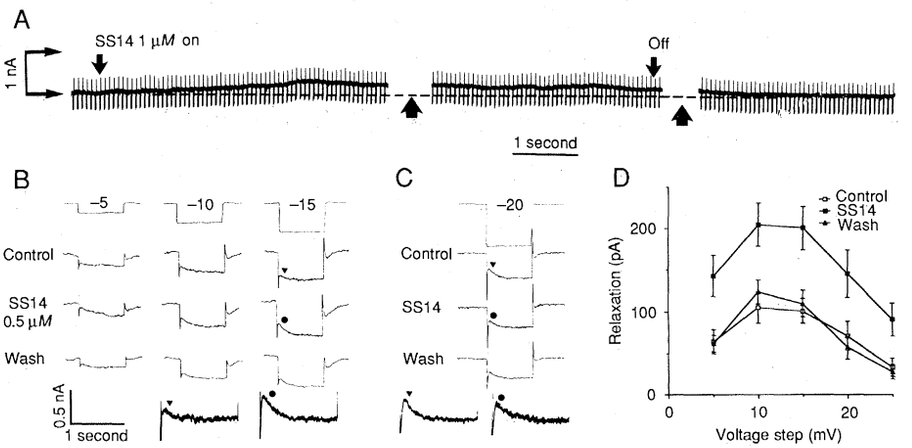

Fig. 1. Effect of SS superfusion on transmembrane currents in CA1 pyramidal neurons: single electrode voltage-clamp data were obtained with the use of potassium chloride–filled micropipettes. TTX was present in all records shown here and in Fig. 2. (**A**) In a pyramidal neuron held at resting potential (−60 mV), SS14 induces an outward (upward) current accompanied by a small increase in ionic conductance, as indicated by the 10 to 15% increase in size of the downward deflections produced by a 10-mV hyperpolarizing command pulse. Upward deflections are due to transient outward currents following command offset (probably I_A). Upward arrows indicate 5-minute gaps in the record during which current-voltage curves were generated. Downward arrows indicate duration of SS14 superfusion. (**B**) In a different neuron, hyperpolarizing voltage commands of 5, 10, and 15 mV (top trace), from a depolarized holding potential (V_H = −40 mV), produce a small inward instantaneous current followed by a slow relaxation to a greater inward steady-state level. The difference between the instantaneous and steady-state currents constitutes the M-current (*8*). Bottom two traces are enlargements (×1.7) of the relaxations obtained from control (triangle) and SS14-treated (circle) conditions during the 15-mV commands; SS14 doubles the M-current. Although only three commands are shown here, command steps of −5, −10, −15, −20, and −25 mV were used in this and all other clamped cells. (**C**) The same neuron held at −65 mV (resting potential) to elicit the Q-current relaxations (*8*) with −20-mV commands (−10- and −30-mV steps gave equivalent results). Somatostatin-14 had no effect on these current relaxations as shown by the enlargements [bottom two traces (×1.7); symbols as in (B)]. (**D**) M-current relaxations from 14 different CA1 pyramidal cells tested with SS14 or SS28 (at 0.5 to 1 μ*M*) and averaged (±SEM) over a range of voltage commands. In all cells V_H was near −40 mV. Somatostatins double the average size of the relaxations but have no detectable effect on the voltage dependency of the relaxations. The extrapolated average apparent threshold potential for these currents was about −70 mV.

−62 mV. Spike size was 75 to 110 mV, and input resistance ranged from 35 to 65 megohms (mean, 50 megohms). As reported (*9–11*), superfusion of SS28 or SS14 elicited hyperpolarizations (in 23 of 30 cells) in association with reduced firing of spontaneous spikes and a small (15 to 30%) reduction in input resistance. No cells were depolarized by SS28 or SS14. Hyperpolarizing concentrations were 0.2 to 1 μ*M*, with maximal effects at 1 μ*M*. SS14 and SS28 were approximately equipotent.

The major ionic candidate for the SS-induced hyperpolarizations is K^+ (*9–11*). To specify which of the K^+ currents might be affected, we tested 19 neurons in voltage-clamp mode [in 0.5 to 1 μ*M* tetrodotoxin (TTX)] for periods of 1 to 4 hours. When neurons were clamped at resting membrane potentials or more positive potentials, SS14 and SS28 superfusion usually (81% of cells) produced a steady outward current of up to 400 pA (mean ± SEM = 190 ± 45 pA) in association with a slight increase in conductance (Fig. 1A). When the membrane potential was held at or near normal resting potentials (−60 to −65 mV), hyperpolarizing voltage steps of 10 to 30 mV elicited slow, inward current "relaxations," termed the Q-current, that were K^+- and Na^+-dependent (*8*). The Q-current (but not the M-current) was blocked by addition of 2 m*M* cesium to the perfusate but was not altered by the superfusion of SS14 or SS28 (five of five cells) (Fig. 1). Therefore, to avoid intermingling of the Q-current with the similar M-current (*8*) (Fig. 1), we routinely added Cs^+ to the superfusate for studies of the M-current.

In hippocampal pyramidal neurons, the M-current is seen with holding potentials of −40 to −50 mV and small hyperpolarizing command steps of 5 to 20 mV and 700 to 1000 msec (*8*) when it appears as a slow inward current relaxation after the instantaneous (ohmic) inward current drop (Fig. 1B) (*8, 18, 20*). As the instantaneous current jump at command onset is larger than that at command offset (Fig. 2), the relaxation represents the slow inactivation of an outward (K^+) current rather than the slow activation of an inward current (*8, 18, 20*). The difference between the instantaneous current at command onset and the steady-state current at the end of the command constitutes the magnitude of the M-current, which in our control recordings reached a maximum of 100 to 300 pA. Tail (off) currents were not analyzed because of contamination with Ca^{2+} and other K^+ currents. The magnitude of the relaxations was voltage-dependent with a maximum amplitude from a holding potential of −40 mV with commands of −10 to −15 mV (Fig. 1D). Superfusion of SS14 or SS28 (0.5 to 1 μ*M*) increased the amplitude of the M-currents by 15 to 480% (mean ± SEM = 197 ± 27%, at −10 mV commands) (Fig. 1D) in 16 of 19 pyramidal neurons. In the three neurons showing no SS effect on the M-current, there was little measurable M-current in the control condition. However, two other cells that had little measurable M-current in control conditions displayed a clear M-current during SS superfusion.

Several lines of evidence suggest that the relaxations enhanced by SS represent the M-current. First, the relaxations were suppressed in five of five cells by superfusion or local pressure application of the muscarinic agonists carbachol (25 or 50 μ*M*) (Fig. 2) or muscarine (25 or 40 μ*M*), as previously described for the M-current in both hippocampal and sympathetic ganglia neurons (*8, 20*). Carbachol (50 μ*M*) completely abolished the current relaxations even during their maximal augmentation by the SSs (Fig. 2). Furthermore, the current relaxations were blocked (in three of three cells) by inclusion of 1 m*M* Ba^{2+} (Fig. 2) in the superfusate, and the current-voltage curves for the relaxations were shifted in a positive direction by an increase of K^+ in the medium from 3.5 to 10.5 m*M* (two of two cells). Thus, the augmenting effect of the SSs on the relaxations likely involves K^+. In addition, the magnitude, kinetics (with approximate time constants of 100 to 200 msec at 28° to 29°C, holding potential V_H = −40 mV, and commands of −10 to −15 mV), and voltage dependence (Fig. 1D) of the relaxations appear equivalent to those of the pyramidal cell M-current (*8*).

We tested the possible effect of SS on other conductances altered by ACh (*4–6*). Afterhyperpolarizations (AHPs), generated by injecting depolarizing current pulses (0.2 to 0.5 nA, 200 to 700 msec in duration) to drive spike trains in current-clamp mode, did not appear to be altered in amplitude or duration by SS14 or SS28 [see figures 4 and 6 of (*9*)]. Furthermore, during superfusion of TTX (0.5 to 1 μ*M*) to block the Na^+ components of the spikes, SS did not measurably alter the size and shape of the presumed Ca^{2+}-dependent spikes and AHPs generated by depolarizing current pulses (Fig. 2C) (four of four cells). In voltage-clamp mode, the various components of the current responses to depolarizing command steps of 5 to 35 mV (200 to 700 msec) from holding potentials of −40 to −60 mV [for example, the transient A-current (*8, 18, 21*) and more prolonged inward and outward currents probably due to activation of Ca^{2+} and other K^+ conductances (*5, 21*)] were also not consistently altered in amplitude or form by SS14 or SS28 (five of five cells).

Thus, SS14 and SS28 specifically enhance a voltage-dependent K^+ current, the M-current. Preliminary voltage-clamp studies of neurons of the solitary tract complex have also shown SS-induced M-current augmentation (*22*). Although many agonists have been shown to reduce the M-current (*5, 7, 8, 18, 20*), to our knowledge this is the first evidence for agonist-induced enhancement of the M-current. These data also show a reciprocal regulation of a single type of voltage-dependent ionic channel by two different transmitters (ACh and SS). However, the SS-muscarinic interaction may be more

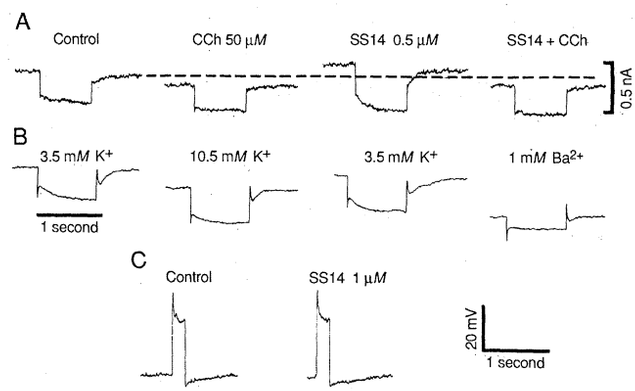

Fig. 2. (**A**) Interaction of SS14 and carbachol (CCh) in voltage-clamp mode. Current records obtained at a holding potential of −40-mV and −15-mV commands (−5-, −10-, −20-, and −25-mV commands not shown). The dashed line indicates the holding current in control conditions for comparison to the effect of CCh (at a maximal concentration), which produces an inward steady current, and to SS14 alone, which produces an outward current. The overall size of the command current (ionic conductance) and the inward M-current relaxation is reduced by CCh but increased by SS14 in the same cell. The ohmic step at pulse onset is larger than that at pulse offset, and this relation persists during superfusion of SS but is lost during superfusion of CCh. However, when added together in the same concentrations, CCh completely overcomes the SS effects. (**B**) A different neuron recorded in voltage-clamp mode: influence of K^+ and Ba^{2+} on the holding current and M-current relaxations. The magnitude of the relaxation is reduced by 10.5 m*M* K^+, and Ba^{2+} nearly abolishes it (holding potential, −40 mV; command steps, −15 mV). Current calibration as in (A). (**C**) Different pyramidal neuron: current-clamp mode in the presence of 1 m*M* TTX. Shown are the lack of effect of SS14 on calcium-dependent spikes, depolarizing responses, and AHPs elicited by intracellular injection of 0.6-nA depolarizing pulses. Resting membrane potential is −57 mV.

complex than simple reciprocal regulation, since in vivo studies indicate that SS alone has predominantly inhibitory actions on pyramidal neurons but is excitatory in the presence of ACh (*13*). This unusual interaction could result from the closing by ACh of the same M-channels opened by SS; in the presence of SS, more M-channels are open for ACh to act upon (perhaps at lower ACh concentrations). Our data (Fig. 2) suggest that ACh will then predominate over the SS effect. However, more direct studies are needed to determine if the other effects of ACh, for example, the voltage-independent depolarizations (*5*) and reduction of Ca^{2+} currents (*6, 23*), are involved in the ACh-SS interaction.

Several other inhibitory agonists (opioid peptides, α-adrenergic agonists, serotonin, baclofen, and adenosine) may also activate voltage-dependent K^+ (inward-rectifying) channels, perhaps through a common mechanism involving a G-protein (*24*). Our studies (*25*) suggest that SS can also open inward-rectifying K^+ channels in hippocampus. Some of the other agonists could also augment the M-current, although our preliminary data on baclofen, serotonin, and adenosine suggest otherwise. In terms of function the M-current will act to clamp the membrane potential at rest (*8, 18*), thus braking regenerative phenomena such as spike bursts. Somatostatin should accentuate this function. Thus, the selective SS-ACh interaction could be involved in epilepsy or in the well-known role of the hippocampus and other cortical areas in behaviors such as memory and learning. The pronounced cortical loss of these two transmitters in dementia of the Alzheimer's type (*26*) further broadens such implications.

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16. Transverse hippocampal slices (from male Sprague-Dawley rats of 100 to 170 g in body weight) 350 to 400 μm thick were cut on a brain slicer, incubated (*15*) and placed in gassed (95% O_2 and 5% CO_2) ACSF that had the following composition, in millimoles per liter: NaCl, 130; KCl, 3.5; NaH_2PO_4, 1.25; $MgSO_4 \cdot 7H_2O$, 1.5; $CaCl_2$, 2.0; $NaHCO_3$, 24; and glucose, 10. Other ions and agents were added to this ACSF medium. The slices were completely submerged and continuously superfused with warm (28° to 37°C) ACSF at a constant rate (2.0 to 4.0 ml/min). Most of the voltage-clamp studies were done at 28° to 29°C to slow M-current kinetics; four cells studied at 35° to 37°C showed the same SS augmentation of the M-current as at lower temperatures. Glass micropipettes filled with potassium chloride (3*M*; tip resistances of 60 to 80 megohms) were used to penetrate CA1 pyramidal neurons. Methods of perfusion, current-clamp recording, cell identification, drug administration, and data analysis were as described (*9, 15*). In the single electrode voltage-clamp mode (*17*), the switching frequency between current injection and voltage sampling was 3 to 4 kHz, and electrode "settling time" was monitored continuously from the headstage input on a separate oscilloscope. For measurement of the instantaneous portion of the M-current with hyperpolarizing commands, the resulting current relaxation was extrapolated back to zero time; this method is valid because of the logarithmic nature of the M-current relaxation (*8, 18*). The various problems (for example, space-clamping) associated with voltage-clamping of neurons with extended processes are discussed elsewhere (*8, 17, 19*). However, these problems are less acute when dealing with relative, qualitative changes after drug or peptide application (*5*), as in the present study.
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A General Method for the Chromosomal Amplification of Genes in Yeast

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The yeast retrotransposon Ty can be used to insert multiple copies of a gene at new sites in the genome. The gene of interest is inserted into a *GAL1*-Ty fusion construct; the entire "amplification cassette" is then introduced into yeast on a high copy number plasmid vector. Transposition of the Ty element carrying the gene occurs at multiple sites in the genome. Two genes, a bacterial neomycin phosphotransferase gene and the yeast *TRP1* gene, were amplified in this way. Although the amplified genes were about 1 kilobase in length, they were amplified to about the same extent as a 40–base pair segment. The benefit of this "shotgun" approach is that amplification can be achieved in one set of manipulations.

YEAST GENOMES TYPICALLY CONTAIN 30 to 40 copies of a transposable element, Ty (*1*). These transposable elements consist of a central region containing two long open reading frames and is flanked by two δ sequences (*2*). New copies of the transposon arise by a replicative transposition process in which the Ty transcript is converted to a progeny DNA molecule by a Ty-encoded reverse transcriptase (*3*). The

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