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Somatostatin selectively enhances acetylcholine-induced excitations in rat hippocampus and cortex

(neurotransmitter interactions/electrophysiology/iontophoresis)

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ABSTRACT **The neuronal effects of somatostatin-14 (SS-14) and its influence on responses to acetylcholine (AcCho) were studied *in vivo* in the rat parietal cortex and dorsal hippocampus, using single-unit recording and microiontophoresis. SS-14 inhibited spontaneous firing of nearly all cells tested, while AcCho facilitated their firing. In contrast to its direct slowing effect, sustained iontophoretic application of SS-14 enhanced AcCho-induced excitations in 78% of all cells tested. This AcCho-enhancing effect of SS-14 was dose dependent. SS-14 did not enhance the responsiveness to pulses of the excitatory amino acid glutamate. Neurons tonically driven by iontophoretic currents of AcCho responded to concurrent pulses of SS-14 with an increase in firing. Thus, iontophoretic application of SS-14 can produce qualitatively different effects on the spontaneous activity of its target cells depending on the simultaneous effects of other chemical messengers. These condition-dependent interactions may explain the diverse neuronal effects of SS-14 reported in the literature.**

The tetradecapeptide somatostatin was originally isolated from extracts of ovine hypothalamus as an inhibitor of growth hormone release from the anterior pituitary (1). Subsequently, it has become evident that this peptide has a widespread distribution throughout the central, peripheral, and gastrointestinal nervous systems and the endocrine pancreas (2–6). Its role as a neurotransmitter in the central nervous system is supported by its presence in neuronal cell bodies and terminals in several regions (2, 7–9), its calcium-dependent release upon depolarization (10–12), and its ability to affect neuronal activity (13–20). Subsequent chemical (5, 6) and cytochemical (8, 21) studies revealed that the cellular localization and the major biological actions of somatostatin are shared with a larger 28 amino acid form, of which the original somatostatin is the C-terminal tetradecapeptide (SS-14). In hippocampus and cerebral cortex, two areas in which somatostatin-containing neurons have been identified in rat and primate (2, 8, 21), SS-14 was originally reported to depress neuronal activity *in vivo* (14). Although many subsequent studies showed predominantly depressant or hyperpolarizing effects of SS-14 in hippocampus (15), hypothalamus (13), and spinal cord (13, 18), other laboratories have reported negligible effects or excitatory or depolarizing actions of SS-14 (16, 17, 19, 20). The source of this range of reported effects is unclear.

Recently, in the course of evaluating the effects of ethanol on the responses of hippocampal pyramidal cells to several neurotransmitters (22), we noticed that SS-14 had inhibitory effects on spontaneous firing unless acetylcholine (AcCho) was included in the multibarrel iontophoresis assembly. In this case, leakage of AcCho was noted to favor excitatory responses to SS-14. Therefore we decided to investigate this phenomenon in more detail. We have now explored the responses of pyramidal cells *in vivo* to the iontophoretic application of either substance alone or the two together, in both the dorsal hippocampus and the parietal cortex, where fields of somatostatin-containing (21, 23) and cholinergic (see, e.g., ref. 24) nerve terminals overlap.

Our studies confirm that SS-14, when applied alone, directly depresses spontaneous discharge rate, with a slow onset and offset of its effects. However, when tested concurrently with either brief pulses or small amounts of AcCho continuously leaked from an iontophoretic pipette, SS-14 caused a dose-dependent enhancement of AcCho-induced facilitations but not those produced by glutamate. Our data could explain the apparently contradictory or inconsistent effects of SS-14 reported by different groups (13–20). These observations (25) may reflect a wider versatility of postsynaptic membrane response mechanisms than is currently acknowledged.

METHODS

Animal Preparation. Male Sprague–Dawley rats (Charles River Breeding Laboratories), 250–350 g in weight, were anesthetized with halothane [2% (vol/vol) in air during surgery and 0.75–1% during experiments] through a tracheal cannula and mounted in a stereotaxic apparatus. After a small portion of bone and dura mater over the parietal cortex had been removed, recording pipettes were lowered into the cortex and areas CA1 and CA3 of the dorsal hippocampus. Stereotaxic coordinates were as follows: A-P 3200 μm; L 3000 μm; V 800–1500 μm for cortex; V 1800–2300 μm for CA1 and V 2800–3500 μm for CA3. Pyramidal cells were identified by their characteristic bursting firing pattern (22), and the recording site was verified by histological localization of a spot of pontamine sky blue ejected from the recording barrel at the end of each experiment.

Electrophysiology and Microiontophoresis. Single-unit activity was recorded through the center barrel of five-barreled micropipette assemblies whose overall tip diameters ranged from 4 to 10 μm. The center barrel was filled with 2% pontamine sky blue in 0.5 M sodium acetate. Three of the outer barrels were filled with drug solutions, while the fourth was filled with 3 M NaCl and used for current neutralization and control. SS-14 (3 mM in normal saline, pH 6.5) was ejected with positive current by electroosmosis and retained between pulses by 5 nA of negative current. AcCho (as the chloride; 0.1 M, pH 4) and scopolamine (2 mM, pH 5) were ejected with positive current and retained with 10–15 nA of negative current. L-Glutamate (0.1 M, pH 8) was ejected with negative current and retained with 10 nA of positive current.

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Abbreviations: SS-14, somatostatin tetradecapeptide; AcCho, acetylcholine.

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All iontophoresis, retention, and automatic neutralization currents were delivered by a BH-2 iontophoresis circuit (Medical Systems, Long Neck, NY).

Action potentials from single pyramidal cells were displayed on an oscilloscope and separated from background activity by a voltage-gating window discriminator, the square pulse output of which was integrated over 1-sec intervals and recorded as firing rate on a polygraph. On-line computer analyses of firing rates and drug effects were performed by a PDP-11/05 MINC computer that generated peridrug response interval histograms (PISH.MAC software of K. Liebold, Research Institute of Scripps Clinic, La Jolla, CA) for subsequent quantification and further analysis. Our criterion for significant drug-induced excitation or inhibition was a sustained change in firing rate of at least 1 standard deviation above or below control baseline firing, as quantified by software (PISHIN, PISHEX) also developed by K. Liebold.

RESULTS

The responses of 58 neurons were studied: 22 were cortical cells, 17 were CA1 pyramidal cells, and 19 were CA3 pyramidal neurons. SS-14 applied alone consistently depressed spontaneous discharge rate (Fig. 1*A*) in about 90% of all cells tested in either hippocampus or cortex. None of the spontaneously firing cells in our experiments showed increased firing when tested with SS-14 alone. The SS-14-induced inhibitions were consistently slow in onset and persisted beyond the offset of the current pulse (Fig. 1*A*).

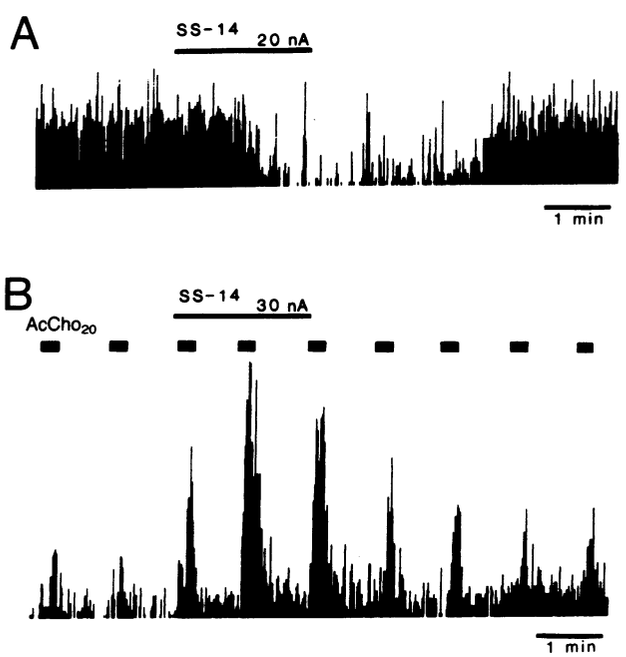

FIG. 1. SS-14 directly depresses spontaneous firing rate but enhances AcCho-induced facilitations. (*A*) The spontaneous activity (displayed as discharge rate) of a single cortical neuron: SS-14 ejected with 20 nA reduces discharge rate. Maximum vertical deflection represents 30 spikes per sec. (*B*) Brief iontophoretic pulses (20 nA) of AcCho ($AcCho_{20}$) facilitate the spontaneous firing of a hippocampal CA1 pyramidal neuron. Responses to AcCho are increased when SS-14 is ejected with 30 nA concurrently from another barrel. The largest vertical deflection represents a firing rate of 72 spikes per sec. In this and all subsequent figures (except 4*B*) vertical deflections proportional to discharge rate were generated by computer, using poststimulus-time histogram software and a single-sweep protocol. Also, bars above records indicate time of drug application.

Tachyphylaxis to the inhibitory effects was noted after repeated SS-14 applications.

By contrast, AcCho facilitated spontaneous firing with a relatively fast time course (Figs. 1*B* and 2–4), as previously reported (ref. 26; see ref. 27 for review). When brief (15- to 20-sec) repetitive iontophoretic pulses of AcCho were applied during a period of sustained exposure (2–3 min) to SS-14, AcCho responses were enhanced (Fig. 1*B*). This effect of SS-14 was observed in 20 of 26 neurons tested (77%), including 8 of 10 (80%) in cortex, 5 of 8 (63%) in CA1, and 7 of 8 (87.5%) in CA3. We term this enhanced responsiveness to AcCho during periods of somatostatin exposure an "enabling" response, in keeping with similar positive conditional or condition-dependent interactions observed for other transmitters such as norepinephrine and vasoactive intestinal peptide (27–31).

Such an enabling effect of SS-14 was not observed with excitatory responses elicited by brief pulses of glutamate (Fig. 2). Rather, SS-14 typically depressed glutamate responses as well as baseline firing, in both cortex and hippocampus. The time course of this depressant action on glutamate excitations was similar to that observed when SS-14 was applied alone to spontaneously active neurons.

The enabling effect of SS-14 on responses to AcCho appeared to be dose dependent in all 15 neurons (6 in cortex,

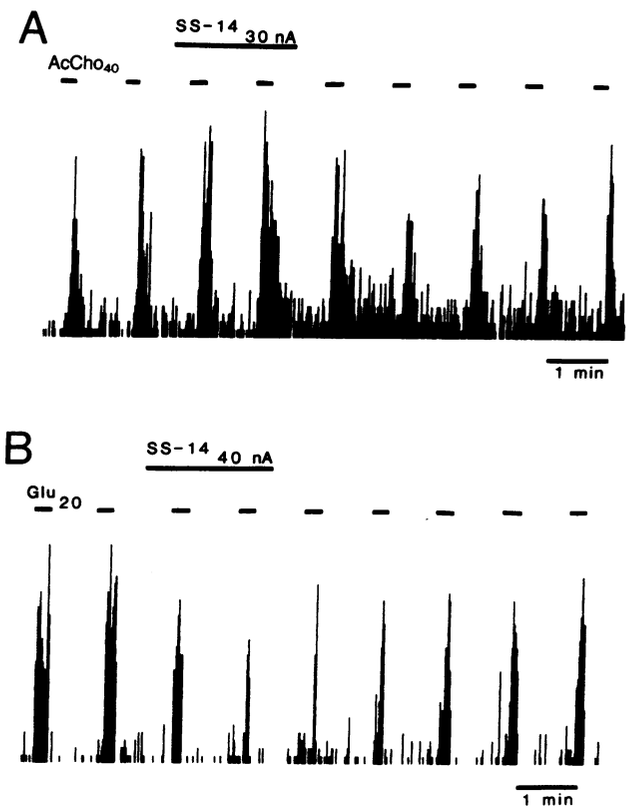

FIG. 2. SS-14 enhances excitatory responses to AcCho but not to glutamate. Both panels show the activity recorded from the same CA1 pyramidal cell of the dorsal hippocampus. (*A*) SS-14 applied iontophoretically (30 nA) enhances excitatory responses to brief (40-nA) pulses of AcCho. The first, second, and third responses to AcCho after the onset of SS-14 current were 164%, 245%, and 196%, respectively, of the average of the first two control AcCho tests. There was no quantitative difference between the first two and last two control AcCho tests illustrated here. Peak vertical deflection = 29 spikes per sec. (*B*) Comparable iontophoretic pulses (20 nA) of glutamate (Glu_{20}) also increase firing, but these responses are not enhanced by concurrent application of SS-14. Rather, SS-14 depresses basal and glutamate-induced firing. Maximum vertical deflection = 28 spikes per sec.

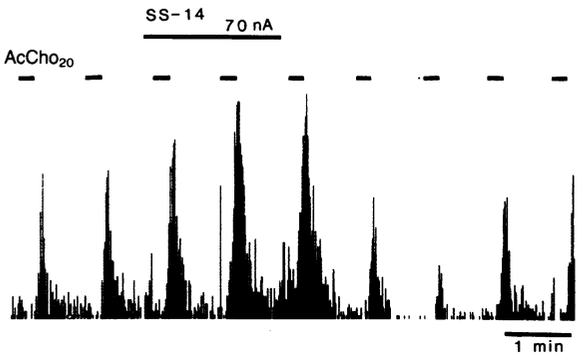

FIG. 3. Effects of SS-14 on AcCho-induced excitations are dose dependent. A CA3 pyramidal cell shows a mixed alteration in responsiveness to AcCho after a 2-min exposure to high currents of SS-14: an initial enhancement of responses to AcCho is followed by a depression of AcCho facilitations and of basal firing. Peak vertical deflection = 59 spikes per sec.

5 in CA1, and 4 in CA3) evaluated with multiple currents of the peptide. At low ejection currents that had little or no direct effect on spontaneous firing rate when tested alone, SS-14 depressed the basal firing between AcCho-induced rate increases but did not affect the magnitude of those increases (data not shown). At greater relative currents of SS-14, or more prolonged currents of lower strength, the predominant effect was a clear increase in the magnitude of responses to AcCho (Fig. 1*B*), which also became slightly longer lasting. Such interactions were consistent and clearcut, with responses to AcCho increasing by more than 100% over control responses, and were reversible within 2–3 min of the termination of SS-14 currents (Fig. 2*A*). At still higher relative currents, SS-14 initially enhanced responses to AcCho, but this enhancement was immediately followed by a depression of both AcCho facilitations and basal firing (Figs. 2*A* and 3).

When the temporal relationship between the application of SS-14 and AcCho was reversed, the enabling effects of the peptide on AcCho responses could still be observed (Fig. 4). Iontophoretic pulses of SS-14 applied to neurons whose firing rate was tonically increased by continuous application of small ("leak") amounts of AcCho caused an increase in neuronal firing in 6 of 8 (75%) cells tested (Fig. 4*B*). The time course of these SS-induced increases in firing was slightly faster than the depressant effects observed when SS-14 was applied alone (this was also the case for the enhancements of AcCho shown in Figs. 1*B* and 3). The effects of SS-14 on AcCho-driven cells were dose dependent (Fig. 4*C*), with high ejection currents producing the same type of biphasic effect as illustrated in Fig. 3. At even higher ejection currents, SS-14 produced only an inhibition of the AcCho-induced tonic discharge. The apparent "excitatory" responses to SS-14 pulses during tonic AcCho activation would seem to be dependent on an activation of a muscarinic cholinergic receptor, as this SS-14 activation was blocked by the muscarinic antagonist scopolamine (n = 2; data not shown).

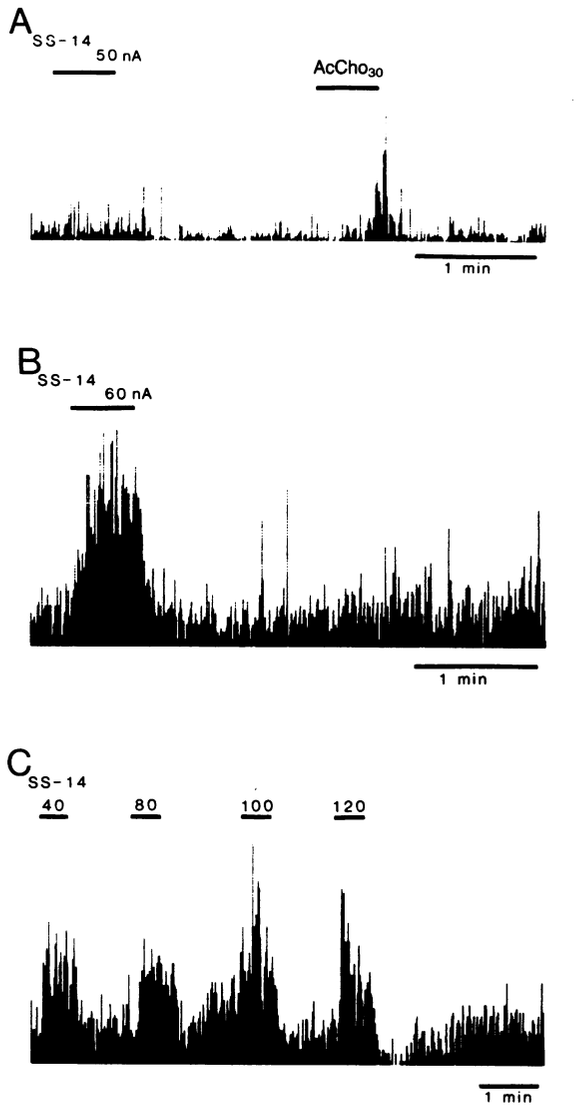

FIG. 4. SS-14 enhances AcCho-induced tonic discharge. (*A*) The spontaneous activity of a CA1 pyramidal cell is inhibited by SS-14 (50 nA) and increased by AcCho (30 nA). Peak vertical deflection = 71 spikes per sec. (*B*) Leakage of AcCho from an iontophoretic pipette by removal of the retaining current tonically increases the discharge rate of the same CA1 cell. Application of SS-14 (60 nA) then results in a brief increase in firing rate. Peak vertical deflection = 58 spikes per sec. (*C*) The effects of SS-14 on AcCho-induced tonic discharge are dose dependent. Higher doses (120 nA) have a biphasic effect similar to that illustrated in Fig. 3*C*. Peak vertical deflection = 59 spikes per sec.

DISCUSSION

Our results confirm several previous reports that SS-14, when applied alone, has predominantly inhibitory actions on the activity of single neurons in the central nervous system (13–15, 18, 27). Our results further indicate that iontophoretic application of somatostatin-14 can yield different, but consistent, effects on postsynaptic cell activity, depending on what other neurotransmitters are being tested simultaneously. For example, SS-14 enhances excitatory actions of AcCho but not those of glutamate. This interaction appears to be selective for SS-14 and AcCho, as there was no enhancement of responses to glutamate, another putative excitatory transmitter. In addition, the qualitative effect produced by SS-14 on neuronal activity in the presence of AcCho depends on the relative dose of the peptide and the test history of the neuron being monitored. As suggested by the results illustrated in Fig. 4, the interaction between the two transmitters seems to be one in which SS-14 enhances the effects of AcCho regardless of the sequence of tests, but not vice versa. High currents of SS-14 can still overcome the AcCho-enhanced responsiveness and produce inhibition of cell firing. During exposure to levels of AcCho that continuously elevate cell firing, low currents of SS-14 now have a predominantly excitatory action. We interpret this latter response as an apparent "excitatory" effect (Fig. 4) due to enabling of the underlying AcCho-induced tonic discharge, since such effects were seen only when cells were tested with low doses

of SS-14 after having been made to fire more rapidly with AcCho. Furthermore, when the muscarinic antagonist scopolamine was used to block effects of AcCho, no subsequent apparent excitatory effects of SS-14 were observed.

We do not yet understand the molecular or cellular mechanisms that underlie the interactions between SS-14 and AcCho. We recognize that the two SS-14 actions could arise from any of several sites (e.g., pre- and postsynaptic) where AcCho is known to act (27). Further studies are required to clarify this possibility. Nevertheless, the observations reported here offer several implications for studies of synaptic mechanisms. First, it is not uncommon that apparent inconsistencies arise between iontophoretic studies aimed at determining the postsynaptic effects of a neurotransmitter within a given synapse (27, 31). For example, the effects of SS-14 in the central nervous system are somewhat controversial, as both inhibitory and excitatory actions have been reported (13–20, 27) even for the same neuron type. Our observations raise the possibility that the reported excitatory actions (16, 17, 19, 20) could have resulted from unrecognized influences of AcCho, either allowed to leak (purposely or inadvertently) from an iontophoretic pipette or released spontaneously from endogenous sources. Thus, in addition to the conventional methodological and interpretative concerns in iontophoretic analysis (32), the possible influence of such dynamic response interactions (27) also must now be considered.

Second, conditional neurotransmitter interactions may also be useful in explaining the actions of drugs on synaptic transmission. For example, we recently found that systemic ethanol selectively enhances responses of hippocampal pyramidal cells to AcCho and SS-14 (22). However, given the interactions between these two substances, the increased responsiveness to AcCho could be an indirect effect of ethanol, resulting from a primary enhancement of the actions of endogenous SS-14.

Third, if the effects of iontophoretic application of SS-14 accurately reflect those of synaptically released SS-14, the peptide could be conceived to have at least two types of conditional effects on hippocampal and cortical neurons. Its "primary" effect, in the absence of AcCho, is to inhibit spontaneous discharge (13–15, 18). In the presence of AcCho, the functional consequences of other conditional effects can vary, depending on the concentration and temporal context of the release of the two substances. As more of the chemical and electrophysiological variables that interact during the normal process of synaptic communication are defined, synaptic processes may emerge as even more complex. SS-14 coexists in synaptic terminals with γ-aminobutyric acid (GABA) (33, 34). Clearly, the effects of coapplication of these two substances with and without AcCho should be explored.

It is becoming increasingly evident that the vocabulary of neuronal communication is much larger and more complex than that previously conceived (27–30). The results presented here suggest that deciphering the grammar of neuronal communication will require careful evaluation of the interactions of transmitters with other transmitters (see refs. 27 and 31), and the temporal context of these interactions, as well as the traditional study of the postsynaptic effects of single transmitters alone.

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