Somatostatin Acts in CA1 and CA3 to Reduce Hippocampal Epileptiform Activity

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Tallent, Melanie K. and George R. Siggins. Somatostatin acts in CA1 and CA3 to reduce hippocampal epileptiform activity. J. Neurophysiol. 81: 1626–1635, 1999. Although the peptide somatostatin (SST) has been speculated to function in temporal lobe epilepsy, its exact role is unclear, as in vivo studies have suggested both pro- and anticonvulsant properties. We have shown previously that SST has multiple inhibitory cellular actions in the CA1 region of the hippocampus, suggesting that in this region SST should have antiepileptic actions. To directly assess the effect of SST on epileptiform activity, we studied two in vitro models of epilepsy in the rat hippocampal slice preparation using extracellular and intracellular recording techniques. In one, GABA-mediated neurotransmission was inhibited by superfusion of the GABA<sub>A</sub> receptor antagonist bicuculline. In the second, we superfused Mg<sup>2+</sup>-free artificial cerebrospinal fluid to remove the Mg<sup>2+</sup> block of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor. We show here that SST markedly reduces the intensity of evoked epileptiform afterdischarges and the frequency of spontaneous bursts in both CA1 and CA3. SST appears to act additively in the two regions to suppress the transmission of epileptiform events through the hippocampus. We further examined SST’s actions in CA3 and found that SST dramatically reduced the frequency of paroxysmal depolarizing shifts (PDSs) recorded intracellularly in current clamp, as well as increasing the threshold for evoking “giant” excitatory postsynaptic currents (EPSCs), large polysynaptically mediated EPSCs that are the voltage-clamp correlate of PDSs. We also examined the actions of SST on pharmacologically isolated EPSCs generated at both mossy fiber (MF) and associational/commisural (A/C) synapses. SST appears to act specifically to reduce recurrent excitation between CA3 neurons because it depresses A/C-evoked EPSCs. SST also increased paired-pulse facilitation of A/C EPSCs, suggesting a presynaptic site of action. Reciprocal activation of CA3 neurons through A/C fibers is critical for generation of A/C EPSCs, suggesting a presynaptic site of action. The actions of SST in the rest of the hippocampus remain uncharacterized. These results indicate concerted in-0022-3077/99 $5.00 Copyright © 1999 The American Physiological Society

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INTRODUCTION

Epilepsy is a disease afflicting ~1% of the U. S. population (Annegers 1994). This disease state is characterized by abnormal hyperexcitability, which can include several different brain regions. Temporal lobe epilepsy (TLE) involves the hippocampus (Sloviter 1994), and treatment for intractable forms of this disorder sometimes involves surgical removal of hippocampal tissue (Sloviter 1994). In such tissue removed from humans with TLE, neurons that contain the neuropeptide somatostatin (SST) are selectively lost in the hilus of the dentate gyrus (de Lanerolle et al. 1989; Mathern et al. 1995; Robbins et al. 1991). Similar results have been found in several animal models of epilepsy (Mitchell et al. 1995; Schwarzer et al. 1995; Sloviter 1987), where SSTergic neuron loss can extend beyond the hilus to the rest of the hippocampus (Lahtinen et al. 1993; Manfridi et al. 1991). The loss of hippocampal neurons is preceded in animal models by increased SST release (Manfridi et al. 1991; Mitchell et al. 1995; Vezzani et al. 1992), suggesting that an early consequence of hippocampal seizures is activation of SSTergic neurons. The function of these SST neurons and the consequences of their loss are unknown. However, the majority of SST interneurons make inhibitory synapses onto primary hippocampal neurons (Freund and Buzsaki 1996; Leranth et al. 1990; Milner and Bacon 1989), suggesting they are involved in inhibitory processes. Thus the death of SST interneurons in early stages of epilepsy may contribute to subsequent abnormal hippocampal hyperexcitability.

In vivo studies examining the effect of SST on seizures induced in rats have produced conflicting results. Early studies using cysteamine to deplete SST suggested a facilitatory role for SST, as cysteamine had anticonvulsant actions when injected intraperitoneally (Higuchi et al. 1983; Perlin et al. 1987). Results with intracerebroventricular injection of anti-SST antibody were similar (Higuchi et al. 1983). However, later studies suggested an antiepileptic role for SST in the hippocampus. Thus an anti-SST antibody perfused directly into the hippocampus enhanced the rate of picrotoxin-induced (Mazarati and Telegdy 1992) or kindling-induced (Monno et al. 1993) seizures in rats. Also SST or its analogs perfused intrahippocampally reduced seizures induced by picrotoxin (Mazarati and Telegdy 1992) or kainate (Perez et al. 1995).

An anticonvulsant action is supported by cellular studies of SST effects in CA1 of hippocampal slices. Here SST hyperpolarizes pyramidal neurons (HPNs) (Pittman and Siggins 1981) by augmenting K<sup>+</sup> currents (Moore et al. 1988; Schweitzer et al. 1988, 1990), resulting in an inhibition of firing. SST also depresses glutamatergic excitatory postsynaptic currents (EPSCs) in CA1 (Tallent and Siggins 1997), probably through presynaptic inhibition of glutamate release (Boehm and Betz 1997). These results indicate concerted inhibitory actions for SST at the cellular level in CA1; we have suggested that this peptide may act as a homeostatic regulator to depress abnormal excitation (Tallent and Siggins 1997). The actions of SST in the rest of the hippocampus remain uncharacterized.
The rat hippocampal slice has been used extensively to study the cellular basis of seizure-like events (Schwartzkroin and Prince 1978, 1980; Traub and Wong 1982). This preparation maintains much intact circuitry, exhibits epileptiform activity in response to various pharmacological manipulations (Mody et al. 1987; Schwartzkroin and Prince 1978; Traub et al. 1994), and offers the advantage that direct effects of exogenous SST can be examined. Therefore we used rat hippocampal slices to study the actions of SST on seizure-like events and to characterize SST actions in CA3, a region critical for the generation of seizure-like events. These are the first studies to directly address the action of SST on epileptiform events in vitro. A preliminary report of some of our findings has been published in abstract form (Tallent and Siggins 1998).

**Methods**

**Slice preparation**

Hippocampal slices were prepared as described previously (Pittman and Siggins 1981; Schweitzer et al. 1993). Briefly, male Sprague-Dawley rats (100–200 g) were anesthetized with halothane (4%) and decapitated, and their hippocampal formation was removed rapidly. We cut transverse slices of 350-μm thickness on a McIlwain brain slicer and placed them in ice-cold (1–3°C) artificial cerebrospinal fluid composition (in mM): 130 NaCl, 3.5 KCl, 1.25 NaH₂PO₄, 1.5 MgSO₄·7H₂O, 2 CaCl₂·2H₂O, 24 NaHCO₃, and 10 glucose. After ~30 min of incubation with their upper surfaces exposed to warmed, humidified carbogen, the slices were submerged completely and continuously superfused with ACSF at a constant rate (2–4 ml/min) for the remainder of the experiment. The inner chamber had a total volume of 1 ml; at the superfusion rates used, 90% replacement of the chamber solution could be obtained within 1–1.5 min. Drugs were added to the bath from stock solutions at known concentrations. We obtained SST from BaChem (Torrance, CA), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) from Tocris Cookson (St. Louis, MO), 2-amino-5-phosphonovaleric acid (APV) from Research Biochemicals International (Natick, MA), and all other chemicals from Sigma (St. Louis, MO). SST was superfused at 1 μM final concentration unless otherwise indicated. We maintained the bath temperature constant at 31°C during testing; for studies examining epileptiform activity in otherwise indicated. We maintained the bath temperature constant at 31°C. For local application of SST, we oriented the slices such that flow through the chamber assisted in limiting diffusion of SST from CA3 to CA1 and vice versa (see Fig. 2A). A glass “puffer” pipette (~20-μm tip diam) was filled with 100 μM SST and lowered close to the surface of the slice. SST was ejected using 1–3 psi pressure. SST application to CA1 involved placing the puffer pipette within 2–4 mm of the recording electrode and applying SST for 10 s. In CA3, we placed the pipette in the stratum radiatum (SR) and applied SST for 20 s. To help ensure that SST reached the neurons from which events were recorded, we placed the recording electrode at a depth of only 50–60 μm into the slice. Three experiments conducted with 0.01% Fast Green in the pipette indicated that ~65–80% of CA3 was perfused; these studies also showed that the Fast Green (and thus probably also SST) was dispersed within a few seconds.

**Extracellular Recording**

We measured both the amplitude and the area (time integral) of the EPSCs in CA3, we evoked EPSCs with paired stimuli applied to the CA3 border in the SR for associational/commissural (A/C) responses (Salin et al. 1996). Stimuli of 0.01- to 0.05-ms duration were applied at 0.16–0.2 Hz, and the data acquisition. We measured PSs from the peak negativity to peak positivity using three or more stimulus intensities to generate input-output (I-O) curves. For normal PSs, intensities were threshold for a consistent PS, half-maximal, and maximal. For evoked bursts, we generally gave five stimulations from threshold to maximal; the maximal stimulus intensity refers to the intensity that generates the greatest number of afterdischarges and the largest burst envelope rather than the maximal initial PS. To maintain stable baseline PSs, we continually delivered SR stimuli at 0.03 Hz.

We evoked epileptiform activity in CA1 and CA3 by stimulating in the SR near the CA1/CA3 border. Bicuculline (15 μM) or Mg²⁺-free ACSF (0 Mg²⁺) was superfused for ≥30 min before the experiment was initiated. We quantified events using coastline burst analysis (CBA)(Korn et al. 1987), which is a measure of the “length” of the outline of the burst waveform. The CBA is calculated by summing the distance between successive points in the digitized burst and subtracting from it an equal duration of a nonbursting recording. This is a sensitive measure of burst intensity and is a useful parameter for determining drug effects (Korn et al. 1987).

**Intracellular recording**

We used discontinuous single-electrode voltage-clamp (switching frequency 3–4 kHz) or current-clamp techniques with sharp intracellular micropipettes (3 M KCl, 50–80 MΩ) as described previously (Tallent and Siggins 1997). To block GABA-mediated inhibitory postsynaptic currents (IPSCs), 10–15 μM bicuculline was included in the bath and, when a GABAₐ component was apparent, 1 μM CGP 55845A. A/C EPSCs were evoked in CA3 HPNs by stimulating in SR near the CA1/CA3 border. To isolate N-methyl-D-aspartate (NMDA) and (R,S)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid/ kainate (AMPA/KA) receptor-mediated EPSCs, we superfused either the NMDA receptor antagonist APV (30 μM) or the AMPA/KA receptor antagonist CNQX (20 μM). We generated MF EPSCs by stimulating in the hilus of the dentate proximal to the inner blade. Because MF EPSCs can be difficult to isolate, we took several precautions to avoid A/C contamination (Claiborne et al. 1993; Williams and Johnston 1991). Thus recordings were done in ACSF containing 7 mM Mg²⁺/4 mM Ca²⁺ and 30 μM APV to block polysynaptic A/C events (Williams and Johnston 1991). We also analyzed only EPSCs with a latency of <4 ms, a rise time <3 ms, and a smooth rising and falling phase (Claiborne et al. 1993; Williams and Johnston 1991). Two traces were averaged for each stimulus intensity. We measured both the amplitude and the area (time integral) of the PSs using Clampfit software (Axon Instruments).

**Statistical analysis**

We performed statistics using two-way ANOVA for repeated measures (Crunch Software, Crunch Software Corporation) with New-
RESULTS

SST effects in CA1

BATH SST SUPERFUSION ATTENUATES EPILEPTIFORM AFTERDISCHARGESRecorded in BICUCULLINE. When PSs were elicited in CA1 in normal ACSF (no drugs, 1.5 mM Mg$_{2}^{+}$), superfusion of 1 µM SST significantly inhibited the PS at all three stimulation intensities $[F(1,7) = 11.90; P < 0.05]$. To determine whether SST could reduce epileptiform activity in CA1, we examined two in vitro epilepsy models. In the first, bicuculline was added to the bath. Bicuculline blocks synaptic inhibition mediated by GABA$_{A}$ receptors, causing the slice to become hyperexcited. Figure 1A shows a normal PS recorded in CA1. After addition of 15 µM bicuculline, a previously submaximal, single stimulus now elicited a burst envelope containing multiple PSs superimposed on an underlying field excitatory postsynaptic potential (EPSP), reflecting abnormal, recurrent firing of CA1 HPNs (Williamson and Wheal 1992). To determine more precisely the action of SST on these epileptiform bursts, we applied five different stimulation intensities to generate I-O curves. As measured by CBI, 1 µM SST significantly and reversibly reduced the intensity of the afterdischarges $[F(1,6) = 16.0, P < 0.05; n = 7; Fig. 1A]$. SST often completely suppressed the afterdischarges at the lowest stimulus intensity, although there was no statistically significant effect of intensity on SST inhibition ($P > 0.1$). SST did not usually affect the number of afterdischarges. In one slice, spontaneous bursts were recorded in the absence of any stimulation; SST completely blocked these spontaneous bursts (not shown).

SST SUPERFUSION DECREASED AFTERDISCHARGESRecorded in Mg$_{2}^{+}$-FREE ACSF. When Mg$_{2}^{+}$ is removed from the superfusate, the Mg$_{2}^{+}$ block of NMDA receptors is relieved and epileptiform events can be evoked (Mody et al. 1987; Traub et al. 1994). These evoked events are similar in shape and intensity to those elicited in bicuculline (Fig. 1B, top). SST reduced the intensity of these evoked afterdischarges $[F(1,6) = 32.29, P < 0.005; Fig. 1B, bottom]$. As with bicuculline, SST effects appeared larger at the lower stimulation intensities, although there was no statistically significant effect of stimulus intensity on the degree of SST inhibition ($P > 0.1$). Figure 1B (top) shows afterdischarges from a representative cell elicited at an intermediate stimulus intensity of 160 µA. SST reversibly reduced the intensity of the afterdischarge.

In the absence of external Mg$_{2}^{+}$, spontaneous discharges driven from CA3 (Mody et al. 1987) were recorded in CA1 (6 of 7 slices). SST reduced the frequency of the spontaneous bursts in five slices; in the other slice with spontaneous discharges, the bursting almost completely disappeared before SST was tested. The control frequency of spontaneous bursting was 0.25 ± 0.05 Hz (ranging from 0.14 to 0.4 Hz). After addition of SST, the frequency fell to 0.07 ± 0.03 Hz (0.01 to 0.2 Hz), representing a 72% inhibition of the bursting frequency $[F(2,8) = 12.8, P < 0.005]$. The effects of SST were reversible; after washout the frequency returned to 0.24 ± 0.04 Hz (0.13 to 0.35 Hz). SST did not noticeably affect the shape or intensity of the spontaneous events.

SST effects in CA3

SST SUPERFUSION REDUCES EPILEPTIFORM EVENTSRecorded in BICUCULLINE. We evoked PSs in CA3 HPNs by stimulating either the MF or the A/C pathways (see METHODS). In contrast to our observations in CA1, 1 µM SST superfused for ≤10 min did not affect the amplitude of MF $[F(1,4) = 0.473, P > 0.5; n = 5]$ or A/C PSs $[F(1,4) = 1.78; P > 0.1; n = 5]$ recorded in normal ACSF. After addition of 15 µM bicuculline to the bath, single, previously submaximal stimuli produced epileptiform afterdischarges. These events were similar to those evoked in CA1 in bicuculline, although they were larger in amplitude and sometimes longer in duration (≤190 ms compared with a maximum duration of 96 ms in CA1). SST (4 min) reduced the intensity of representative afterdischarges (Fig. 2A) evoked by an intermediate stimulation intensity (stimulus 4 in Fig. 2B). SST significantly attenuated the intensity of the afterdischarges as determined by CBI $[F(1,6) = 12.2, P < 0.05; n = 7; Fig. 2A]$. In contrast to CA1, in CA3 a significant interaction of stimulus intensity and SST inhibition was observed, with a greater inhibition at the lower two stimulus intensities $[F(4,24) = 6.75, P < 0.001]$. SST reduces epileptiform events induced in Mg$_{2}^{+}$-FREE ACSF. In the absence of extracellular Mg$_{2}^{+}$, epileptiform events could be evoked at previously submaximal stimulus intensities. The

man-Keuls post hoc test when appropriate. Data are reported as means ± SE and considered statistically significant at $P < 0.05$. 

FIG. 1. Somatostatin (SST) reduces epileptiform activity recorded extracellularly in CA1. A: SST effects in bicuculline. Top: CA1 population spike (PS) generated by stimulation of the Schaeffer collaterals (SCs). After superfusion of 15 µM bicuculline, an epileptiform discharge was generated by a previously submaximal stimulus (220 µA). Superfusion of 1 µM SST caused a robust attenuation of the afterdischarge that recovered on washout. In this and subsequent figures, point of stimulation. Stimulus artifacts in this and subsequent figures are blanked partially and/or truncated. Bottom: mean reduction by SST (n = 7) of CA1 epileptiform burst intensity in bicuculline as measured by coastline burst index (CBI) across a range of 5 stimulus intensities; SST’s actions were reversible on washout. B: SST CA1 effects in Mg$_{2}^{+}$-free artificial cerebrospinal fluid (ACSF). Top: evoked CA1 epileptiform bursts recorded in Mg$_{2}^{+}$-free ACSF; 1 µM SST reversibly reduces the afterdischarge evoked at normal stimulus intensity 4 (see graph, bottom). Bottom: mean effect of SST (n = 7) on CA1 epileptiform events evoked in Mg$_{2}^{+}$-free ACSF, showing a mean 40–60% reduction in CBI over the range of stimulus intensities.
representative afterdischarges in Fig. 2B show that SST (1 μM, 4 min) can completely block the epileptiform events evoked at a threshold stimulus intensity. I-O curves generated from 6 slices show significant reduction of burst intensity by SST [F(1,5) = 34.43, P < 0.005], with a significant interaction between stimulus intensity and SST inhibition [F(4,20) = 5.76, P < 0.005]. SST also significantly reduced the frequency of spontaneous bursting recorded in CA3 [F(2,10) = 38.8, P < 0.0001; Fig. 2C], as in CA1, from 0.28 ± 0.04 Hz (range 0.17 to 0.45 Hz) to 0.08 ± 0.02 Hz (0.0 – 0.15 Hz; n = 7), representing a 70% reduction in rate of bursting. In two of the slices, SST completely suppressed the spontaneous activity. After washout of SST, bursting frequency returned to 0.27 ± 0.04 Hz (range 0.18 – 0.42 Hz). A higher concentration of SST (5 μM) reduced the frequency of spontaneous bursts by 80 ± 4% (n = 4) while 100 nM SST caused a 60 ± 7% decrease (n = 6). SST did not appear to consistently affect the shape or intensity of the spontaneous bursts.

Effects of locally applied SST

CA1 epileptiform events require input from CA3 (Schwartzkroin and Prince 1978). Stimulating near the CA1/CA3 border evokes firing in CA1 both by directly activating Schaeffer collaterals (SCs) and by indirectly activating SCs by exciting CA3 neurons through stimulation of A/C fibers. Thus transecting SC input from CA3 with a knife cut greatly curtailed epileptiform events evoked in CA1 (not shown). Therefore, although SST is known to act directly on CA1 HPNs (Moore et al. 1988; Pittman and Siggins 1981; Schweitzer et al. 1993), it also could act in CA3 to reduce CA1-recorded afterdischarges. SST effects on evoked epileptiform events appear more robust in CA1 than CA3, especially at higher stimulation intensities, suggesting that SST may act additively in CA1 and CA3 to reduce CA1-recorded events. To resolve more discretely the SST site of action, we examined epileptiform events evoked in bicuculline with SST applied locally either to CA1 near the recording electrode or to CA3 (Fig. 3A).

LOCAL SST REDUCES PSs IN CA1. We first determined whether puffer application of SST close to the CA1 recording electrode could reduce PSs recorded in normal ACSF. A 10-s application of SST significantly reduced the amplitude of the PS [F(1,4) = 13.2, P < 0.05, Fig. 3B; n = 5]. There was no significant interaction between stimulus intensity and SST’s effect (P > 0.5). Complete recovery took 5 –10 min in four of the five slices; one slice took 20 min to return to control values. Puffer application of ACSF alone close to the recording electrode did not significantly affect mean CA1 PS amplitudes [F(1,2) = 0.07, P > 0.5; n = 3; Fig. 3B], nor did a 20-s application of SST to the CA3 [F(1,3) = 0.014, P > 0.5, n = 4, Fig. 3B].

LOCAL SST REDUCES BICUCULLINE-INDUCED EPILEPTIFORM EVENTS IN CA1. To determine whether application of SST just to CA1 was sufficient to reduce the intensity of afterdischarges recorded in bicuculline, we locally applied 100 μM SST near the recording electrode and evoked burst afterdischarges at three different stimulation intensities. SST robustly inhibited the epileptiform event across the range of intensities [F(1,4) = 25.8, P < 0.01; Fig. 3C]. Recovery occurred after 6 –13 min of washout. As with bath superfusion, at the lowest stimulation intensity local SST sometimes completely blocked the afterdischarge (Fig. 3C, top). However, the mean decrease in CBI at the minimal stimulation intensity was only to 42 ± 8.1% of control with local SST (Fig. 3C, bottom) compared with 19.8 ± 10% of control with bath perfusion (Fig. 3A, bottom). Although it is unclear whether a maximal equilibrium concentration of SST is reaching the relevant neuronal populations with short puffer application (even with a high SST concentration in the pipette), this difference in effect also could be due to additive actions of SST in both CA3 and CA1 with bath perfusion. Puffer application of ACSF alone to CA1 (n = 3; Fig. 3B, bottom) did not significantly alter the intensity of epileptiform bursts [F(1,2) = 0.197, P > 0.5].

LOCAL SST IN CA3 REDUCES EPILEPTIFORM EVENTS RECORDED IN CA1. To determine if direct actions of SST in CA3 alone could influence epileptiform events recorded in CA1, we locally applied 100 μM SST to CA3 positioned “downstream” from CA1 to limit possible diffusion of SST to the CA1 (Fig. 3A). A 20-s application of SST to CA3 caused a modest but
significant reduction of epileptiform intensity recorded in CA1 [F(1,4) = 39.4, P < 0.005, n = 5; Fig. 3C], with no significant effect of stimulus intensity (P > 0.1). Recovery occurred in 4–8 min in three slices and in 15–20 min in the other two. With the same pressure and pipettes, the degree of inhibition was less than that produced by local application of SST to CA1 (Fig. 3C, bottom). The representative recording illustrated in Fig. 3C (top) shows that even at the lowest stimulation intensity a complete reduction of the epileptiform event did not occur. ACSF alone applied to CA3 did not effect CA1 epileptiform events (n = 3; Fig. 3C, bottom). Thus SST applied to CA3 can depress epileptiform events recorded in CA1, although to a lesser degree than local CA1 or bath application.

**SST effects on intracellularly recorded CA3 events**

SST reduces paroxysmal depolarizing shifts and giant EPSCs in CA3 neurons. Paroxysmal depolarizing shifts (PDSs) recorded in HPNs in current-clamp mode are the intracellular correlate of spontaneous epileptiform bursts recorded extracellularly (Johnston and Brown 1984; Traub et al. 1994). These events are driven polysynaptically (Wong and Traub 1983) and consist of a large EPSP underlying a burst of action potentials. The PDSs contain both NMDA and AMPA/KA glutamate receptor-mediated components when generated in Mg²⁺-free ACSF (Mody et al. 1987; Traub et al. 1994) or bicuculline (Dingledine et al. 1986). In our intracellular studies, SST reduced the frequency of CA3 PDSs recorded in Mg²⁺-free ACSF (Fig. 4A) from a control rate of 0.22 ± 0.02 Hz to 0.09 ± 0.02 Hz (n = 4), with recovery on washout (0.26 ± 0.04 Hz). Although individual bursts were quite variable, we observed no significant alteration of their shape or size by SST.

In voltage-clamp mode, PDSs appear as “giant” EPSCs of 1- to 3-nA amplitude. These EPSCs are not graded but evoked in an “all-or-none” manner (Ben-Ari and Gho 1988). We evoked giant EPSCs in CA3 HPNs by stimulating the A/C pathway in Mg²⁺-free ACSF and bicuculline. We also raised extracellular Ca²⁺ by 3–5 mM to prevent spontaneous bursts that were difficult to voltage-clamp adequately. SST completely blocked the giant EPSCs (Fig. 4B; n = 4). Increasing the stimulus strength could again evoke giant EPSCs with a shape essentially unchanged from the controls (Fig. 4B). After washout of SST, superfusion of 30 μM APV blocked the giant EPSC. Increasing the stimulus strength again evoked a giant EPSC but with a much briefer time course than in the control, indicating that the late component of the EPSC was NMDA-receptor dependent. Subsequent superfusion of CNQX completely blocked the remaining EPSC (not shown); this effect could not be overcome by further increasing the stimulus strength. These results indicate that giant EPSCs are driven by both NMDA and AMPA/KA receptor activation. SST appears to decrease the excitatory drive generating the giant EPSCs and does not appear to affect preferentially the different glutamate pathways in Mg²⁺-free ACSF and bicuculline. We also raised extracellular Ca²⁺ by 3–5 mM to prevent spontaneous bursts that were difficult to voltage-clamp adequately. SST completely blocked the giant EPSCs (Fig. 4B; n = 4). Increasing the stimulus strength could again evoke giant EPSCs with a shape essentially unchanged from the controls (Fig. 4B). After washout of SST, superfusion of 30 μM APV blocked the giant EPSC. Increasing the stimulus strength again evoked a giant EPSC but with a much briefer time course than in the control, indicating that the late component of the EPSC was NMDA-receptor dependent. Subsequent superfusion of CNQX completely blocked the remaining EPSC (not shown); this effect could not be overcome by further increasing the stimulus strength. These results indicate that giant EPSCs are driven by both NMDA and AMPA/KA receptor activation. SST appears to decrease the excitatory drive generating the giant EPSCs and does not appear to affect preferentially the different glutamate pathways in Mg²⁺-free ACSF and bicuculline.
receptor subtypes, consistent with a presynaptic site of action. Furthermore SST induced a only small outward current in two of the four cells (10 –30 pA, V_H = –74 ± 1 mV); in the other two cells (including the one shown in Fig. 4B), SST had no detectable postsynaptic effect. Thus there was no correlation between the amplitude of the SST-induced outward current and SST depression of the giant EPSC, further supporting a presynaptic site of action for SST.

SST EFFECTS ON ISOLATED CA3 EPSCs. To more precisely determine SST’s effects on synaptic transmission in CA3, we recorded intracellularly from CA3 HPNs and evoked EPSCs by stimulating either mossy or A/C fibers. MF EPSCs were isolated in the presence of APV (30 μM), to block the NMDA neurotransmission largely derived from A/C synapses (Claiborne et al. 1993). Interestingly, monosynaptic MF EPSCs were insensitive to SST (Fig. 5A), even though SST induced an outward current in four of the five cells (10–30 pA, V_H = –74 ± 1 mV). In contrast, SST significantly reduced both the amplitude and area of both AMPA/KA and NMDA EPSCs generated at A/C synapses (Fig. 5B and C). Digital subtraction of the SST-insensitive EPSC from the control EPSC revealed that the SST-sensitive component of these currents appeared to be largely polysynaptic (Fig. 5, B and C, top) as suggested by the relatively long latency and slow rise-time of the waveforms (see Berry and Pentreath 1976; Claiborne et al. 1993). SST induced an outward current in 6 of the 10 neurons from which A/C generated EPSCs were recorded, ranging from 10–30 pA (V_H = –75 ± 1 mV). Again, no correlation was found between the SST-induced outward current and SST inhibition of the EPSCs.

To further determine the site of action for SST in inhibiting A/C EPSCs, we examined paired-pulse (P-P) modulation of NMDA EPSCs. Figure 5D, top, shows representative paired NMDA EPSCs evoked at an interstimulus interval (ISI) of 100 ms. SST potentiated the degree to which the second EPSC was potentiated relative to the first EPSC. Figure 5D (bottom) shows the mean results from five (ISI = 200 ms) or six (ISI = 100 and 300 ms) neurons. SST enhanced P-P modulation at all three stimulus intensities; indeed, at the 300 ms ISI, SST shifted a P-P inhibition (the 2nd EPSC was 87 ± 6% of the 1st
EPSC) to P-P potentiation (108 ± 7%). These results further suggest that SST is acting presynaptically to inhibit glutamate release (Manabe et al. 1993; Schulz et al. 1994; Zucker 1973).

**DISCUSSION**

We have shown that SST has robust antiepileptic properties in both CA1 and CA3 regions of the hippocampus. The cellular actions of SST in CA1 have been well characterized. SST augments two types of K⁺ currents in CA1 HPNs, the M current (Moore et al. 1988; Schweitzer et al. 1990, 1993) and a voltage-insensitive leak current (Schweitzer et al. 1998). SST also depresses glutamatergic EPSCs in CA1 HPNs (Tallent and Siggins 1997), probably through a presynaptic mechanism (Boehm and Betz 1997). All these actions are inhibitory in that they reduce the likelihood of a neuron firing an action potential.

**SST superfusion inhibits CA1 but not CA3 PSs recorded in normal ACSF**

SST superfusion reduced normal PSs recorded in CA1, as previously reported (Watson and Pittman 1988). These results indicate that SST decreased the number of neurons that fired at a given stimulus strength. Because SST reduces the probability of neuronal firing by postsynaptic augmentation of K⁺ currents (Moore et al. 1988; Schweitzer et al. 1998) and also reduces presynaptic glutamate release (Boehm and Betz 1997), the postsynaptic actions of superfused SST synergistically act to reduce PSs. In contrast, SST superfusion did not reduce normal PSs recorded in CA3 at either MF or A/C synapses. Although the postsynaptic actions of SST on CA3 neurons have not been characterized, in the present study, SST induced only weak outward currents in CA3 neurons from which synaptic events were recorded. For example, at −70-mV holding potentials, SST induced an outward current of 10–30 pA compared with a mean of 50 pA reported for CA1 neurons (Schweitzer et al. 1993). Therefore SST may have weaker postsynaptic effects in CA3 than in CA1, perhaps accounting for the lack of SST actions on normal CA3 PSs. However, we did not examine postsynaptic effects of SST in the depolarized range, where its actions on the voltage-sensitive M current would be more apparent (Moore et al. 1988).

**SST superfusion reduces epileptiform events in CA1**

In the bicuculline "seizure" model, all synaptic inhibition mediated by GABA_A receptors is blocked, whereas excitatory events are normal (Schwartzkroin and Prince 1980). In normal extracellular K⁺ (3.5 mM), spontaneous epileptiform "interictal" or "preictal" events do not generally occur in CA1 or CA3 (McNamara 1994). However, afterdischarges are elicited at relatively low stimulus intensities. In the other model used (Mg²⁺-free ACSF), the Mg²⁺ block of NMDA receptors is removed, increasing the excitatory drive onto HPNs (Traub et al. 1994); inhibitory pathways are largely unaffected (Mody et al. 1987; Traub et al. 1994). Spontaneous bursting is generated in CA3 and transmitted to CA1 via Schaeffer collaterals (Mody et al. 1987). We used these two different models to determine whether SST effects were similar under these different conditions.

In CA1 in bicuculline, SST often completely suppressed the epileptiform afterdischarges elicited at low stimulation intensities, thus shifting the threshold for evoking an afterdischarge to a higher stimulus strength. Inhibition of epileptiform events peaked within 2–4 min of onset of SST perfusion and was reversible on washout. SST had similar effects in CA1 in the Mg²⁺-free ACSF model. Here, the spontaneous interictal or preictal bursts at the cellular level are composed of synchronized bursting of neuronal populations (Traub and Wong 1982). SST markedly decreased the frequency of this spontaneous bursting in both CA1 and CA3 while not consistently affecting the shape or intensity of the individual bursts. Synchronization of interictal events may lead to ictal or seizure episodes (Traynelis and Dingledine 1888; Williamson et al. 1995). Thus suppression of interictal event frequency by SST could have a protective effect, preventing the onset of seizure activity by reducing the probability of synchronization.

**SST superfusion reduces epileptiform events in CA3**

In CA3, SST effectively reduced or blocked afterdischarges evoked in both bicuculline and Mg²⁺-free ACSF. SST's actions in CA3 under either condition were qualitatively similar to those in CA1 but were less intense, especially at higher stimulation intensities. Thus in CA3, but not CA1, SST effects could be overcome by increasing stimulation intensity. At the cellular level, this suggests that glutamate release in CA3 is sufficient at more intense stimuli to compensate for presynaptic inhibition and/or postsynaptic hyperpolarization by SST. As noted above, SST's postsynaptic actions in CA3 may be weaker than in CA1. Further there appears to be less expression of SST receptors in CA3 than in CA1 (Leroux et al. 1993; Martin et al. 1991).

More robust SST effects in CA1 also may reflect additive actions in CA3 and CA1. Generation of seizure-like events occurs in CA3, through synchronization of CA3 HPN firing via feedforward recurrent excitatory axon collaterals between these primary neurons (Traub and Wong 1982; Wong and Traub 1983). Transecting SC input blocks the invasion of seizures from CA3 into CA1 (Schwartzkroin and Prince 1978). Furthermore, stimulation of SC under epileptiform conditions activates CA1 neurons both directly and indirectly by firing CA3 neurons. Therefore with bath superfusion, direct actions of SST in both CA1 and CA3 could markedly affect epileptiform events recorded in CA1. We addressed this issue by applying SST locally to either CA1 or CA3 while recording CA1 PSs or afterdischarges evoked in bicuculline. SST applied locally near the recording electrode robustly decreased both normal PSs and epileptiform afterdischarges. SST applied in CA3 also could significantly reduce CA1 afterdischarges, while having no effect on normal CA1 PSs. Thus SST can have effects in CA1 when applied only to CA3, although its actions are less pronounced than when applied locally to CA1 or in the superfusate. SST therefore can act directly in both CA3 and CA1 to reduce the spread of epileptiform events through the hippocampus.

**SST selectively reduces excitatory transmission at A/C synapses**

SST dramatically reduced the frequency of PDSs recorded intracellularly in CA3 neurons. PDSs are generated when A/C
synapses, which form recurrent excitatory connections between CA3 HPNs, are unmasked (Traub et al. 1994). Recorded in voltage-clamp, PSDs are seen as nongraded giant EPSCs (Ben-Ari and Gho 1988) that contain both NMDA and AMPA/KA components (Dingledine et al. 1986; Mody et al. 1987; Traub et al. 1994). SST increased the threshold for evoking giant EPSCs without affecting their basic shape, suggesting that SST does not preferentially act on the NMDA or AMPA/KA component. These results are consistent with a presynaptic action on glutamate release. Interestingly, SST was ineffective in depressing monosynaptic MF EPSCs, suggesting that SST may not act on primary synaptic transmission between the dentate and CA3. However, SST did depress both pharmacologically isolated NMDA and AMPA/KA receptor-mediated EPSCs generated at A/C synapses, further supporting a presynaptic site of action. We observed a greater effect of SST on isolated NMDA EPSCs compared with AMPA/KA EPSCs, especially at the highest stimulus intensity (Fig. 5, B and C, bottom), that may reflect the recruitment of more polysynaptic pathways (Crepel et al. 1997). NMDA receptors make a larger contribution at recurrent excitatory synapses between HPNs in both CA1 and CA3 than at primary MF/CA3 (Zulutschy and Nicoll 1990) or SC/CA1 (Deuchars and Thomson 1996) synapses. Furthermore, when isolated, NMDA receptors are more efficient at generating polysynaptic transmission than AMPA/KA receptors (Crepel et al. 1997) and are increased in epileptic hippocampus (Ashwood and Wehail 1986; Mody and Heinemann 1987; Turner and Wehail 1991). These results suggest a selective action for SST in CA3 on the recurrent feedforward excitatory synapses that generate epileptiform activity (see Boehm and Betz 1997).

We further characterized SST actions on A/C EPSCs in CA3 by examining P-P modulation of NMDA EPSCs, whereby facilitation or inhibition of the second of a pair of EPSCs by the first, conditioning EPSC, is measured. An inverse relationship exists between degree of facilitation and glutamate release (Manabe et al. 1993; Schulz et al. 1994; Zucker 1973). Thus an increase in P-P facilitation by a drug suggests presynaptic inhibition of glutamate release. SST enhanced P-P facilitation of A/C-generated NMDA EPSCs, suggesting that SST may not significantly alter primary hippocampal synaptic transmission along the major trisynaptic pathway but that aberrant or recurrent excitation is needed for unmasking of SST-sensitive synapses in both CA1 and CA3. This also is suggested by our findings that SST has no effect on CA3 PSs elicited in normal ACSF but inhibits both spontaneous and evoked epileptiform events recorded in Mg2+-free ACSF and bicuculline. However, SST (both locally applied and superfused) does depress normal PSs in CA1 as well as some apparent monosynaptic NMDA and AMPA/KA EPSCs (Tallent and Siggins 1997), suggesting that in CA1 SST also can affect primary synaptic transmission. However, its CA1 effects appear to be amplified when recurrent excitation is unmasked (Tallent and Siggins 1997).

Interestingly, postsynaptic effects of SST in CA1 are greatest when the neuron depolarizes, because SST augments the outwardly rectifying K+ current (Moore et al. 1988; Schweitzer et al. 1993). Thus SST effects in hyperexcited hippocampus may be increased both by unmasking of latent SST-sensitive recurrent excitatory synapses and by depolarization of HPNs during the PDS. Endogenous release of peptides appears to require high-frequency activation of the peptidergic neuron (Hokfelt 1991), such as might occur during an epileptiform burst (Vezzani et al. 1992). Therefore in epileptic hippocampus may be increased both by unmasking of latent SST-sensitive recurrent excitatory synapses and by depolarization of HPNs during the PDS. Endogenous release of peptides appears to require high-frequency activation of the peptidergic neuron (Hokfelt 1991), such as might occur during an epileptiform burst (Vezzani et al. 1992). Therefore in epileptic
pocampus, conditions for SST release would be matched with increased SST efficacy. This may be an especially important homeostatic mechanism given the collapse of GABAergic inhibition during high-frequency firing (Le Beau and Alger 1998). This apparent selective action of SST on feedforward excitation is unique from that of other neuropeptides, such as neuropeptide Y and the opioids, that have much greater effects on standard hippocampal neurotransmission (Capogna et al. 1993; Klapstein and Colmers 1993).

Recurrent feedforward excitation may be increased in animal models of epilepsy. In addition to the MF sprouting that occurs in the dentate in these models (de Lanerolle et al. 1989; Tauck and Nadler 1985), recent studies suggest that sprouting of local axon collaterals occurs in CA1 as well (Perez et al. 1996). These collaterals appeared to form synaptic contacts with HPN dendrites and also may have formed autapses (Perez et al. 1996). Autaptic EPSCs are SST sensitive in cultured hippocampal neurons (Boehm and Betz 1997). Electrophysiological evidence of increased recurrent excitation in CA1 has been suggested by the appearance of prolonged synchronous afterdischarges in isolated CA1 from kainate-treated rats (Meier and Dudek 1996; Meier et al. 1992). The increase of these SST-sensitive synapses in animal models of epilepsy and the fact that SST receptors largely are spared or even upregulated in epileptic tissue (Perez et al. 1995; Ptwko et al. 1996; Robbins et al. 1991) indicates that SST receptors could be significant pharmacological targets for clinical treatment of epileptic disorders.

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