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Somatostatin type-2 receptor activation inhibits glutamate release and prevents status epilepticus

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ABSTRACT

Newer therapies are needed for the treatment of status epilepticus (SE) refractory to benzodiazepines. Enhanced glutamatergic neurotransmission leads to SE, and AMPA receptors are modified during SE. Reducing glutamate release during SE is a potential approach to terminate SE. The neuropeptide somatostatin (SST) is proposed to diminish presynaptic glutamate release by activating SST type-2 receptors (SST2R). SST exerts an anticonvulsant action in some experimental models of seizures. Here, we investigated the mechanism of action of SST on excitatory synaptic transmission at the Schaffer collateral-CA1 synapses and the ability of SST to treat SE in rats using patch-clamp electrophysiology and video-EEG monitoring of seizures. SST reduced action potential-dependent EPSCs (sEPSCs) at Schaffer collateral-CA1 synapses at concentrations up to 1 μM; higher concentrations had no effect or increased the sEPSC frequency. SST also prevented paired-pulse facilitation of evoked EPSCs and did not alter action-potential-independent miniature EPSCs (mEPSCs). The effect of SST on EPSCs was inhibited by the SST2R antagonist cyanamid-154806 and was mimicked by the SST2R agonists, octreotide and lanreotide. Both SST and octreotide reduced the firing rate of CA1 pyramidal neurons. Intraventricular administration of SST, within a range of doses, either prevented or attenuated pilocarpine-induced SE or delayed the median time to the first grade 5 seizure by 11 min. Similarly, octreotide or lanreotide prevented or attenuated SE in more than 65% of animals. Compared to the pilocarpine model, octreotide was highly potent in preventing or attenuating continuous hippocampal stimulation-induced SE in all animals within 60 min of SE onset. Our results demonstrate that SST, through the activation of SST2Rs, diminishes presynaptic glutamate release and attenuates SE.

Introduction

Novel therapies are needed for the treatment of status epilepticus (SE), which is currently treated with benzodiazepines, barbiturates, and anesthetics such as propofol and midazolam, all of which enhance GABAergic inhibition (Meierkord et al., 2010). However, GABAergic inhibition is compromised during SE (Goodkin et al., 2005, 2008; Kapur and Macdonald, 1997; Naylor et al., 2005), and pharmacoresistance to benzodiazepines develops (Meierkord et al., 2010; Neligan and Shorvon, 2010; Treiman et al., 1998). An alternate approach to treat SE is to reduce excitatory neurotransmission. The activation of muscarinic receptors increases glutamate release from presynaptic terminals of hippocampal principal neurons (Kozhemyakin et al., 2010; Olivos and Artalejo, 2008; Sun and Kapur, 2012) and can cause SE in humans and experimental animals (McDonough and Shih, 1997; Okumura et al., 1996; Turski et al., 1989). Furthermore, AMPA receptors (AMPARs) expressed on CA1 pyramidal neurons of the hippocampus are modified during SE (Rajasekaran et al., 2012). The blockade of AMPARs can terminate benzodiazepine-refractory SE (Fritsch et al., 2010; Rajasekaran et al., 2012), suggesting that inhibiting postsynaptic excitatory receptors can terminate SE. A similar approach to reduce the strength of glutamatergic transmission during SE is to reduce the release of neurotransmitter.

Somatostatin (SST) is proposed to reduce glutamatergic neurotransmission (Boehm and Betz, 1997). However, studies on SST and glutamatergic neurotransmission were performed on cultured hippocampal neurons at autaptic synapses (Boehm and Betz, 1997), which do not occur in intact hippocampal preparations. Furthermore, it is difficult to clearly differentiate between pre- and post-synaptic effects in autaptic preparations. The actions of SST are mediated by SST receptor (SSTR) subtypes 1–5, which are widely distributed throughout the brain (Hoyer et al., 1995; Schulz et al., 2000). Immunocytochemical studies demonstrate that SST2R is located on the soma, dendrites, and axons of hippocampal neurons, suggesting that SST2R may mediate both the presynaptic and postsynaptic effects of SST (Dournaud et al.,

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1996). Indeed, SST2R agonists mimic and antagonists block the inhibitory effect of SST on glutamate release from cortical synaptosomes (Grilli et al., 2004). However, whether SST2R mediates the effects of SST at hippocampal synapses remains unexplored.

SST suppresses kindled seizures (Mazarati and Telegdy, 1992; Mazarati and Wasterlain, 2002; Monno et al., 1993; Zafar et al., 2012) and brief epileptiform discharges in hippocampal slices (Tallent and Siggins, 1999). These anticonvulsant actions are proposed to be mediated by SST2R and SST4R in a species-specific manner (Aourz et al., 2011; Cammalleri et al., 2004; Moneta et al., 2002; Qiu et al., 2008; Stragier et al., 2006; Vezzani et al., 1991). However, SE differs from isolated seizures because the prolonged seizures of SE cause rapid plasticity of neurotransmission. There is some evidence that SST could transiently terminate electrically-induced SE (e. g. Mazarati and Wasterlain, 2002); however, the efficacy of SST and its analogs in terminating SE is not well characterized.

We tested whether SST suppresses glutamate release by activating SST2Rs. Further, we tested the ability of SST and SST2R agonists to treat SE.

Methods

All experimental procedures were performed according to a protocol approved by the University of Virginia Animal Care and Use Committee. Adult male Sprague–Dawley rats (220–250 g, 60–90 days) were used for the experiments.

Electrophysiology

The animals were anesthetized with isoflurane prior to decapitation. Brains were removed and immersed in cold (2–4 °C) slicing ACSF composed of (in mM) 5.5 NaCl, 2 KCl, 5 $MgSO_4$, 1.1 KH_2PO_4, 1 $CaCl_2$, 10 dextrose, 25 $NaHCO_3$ and 113 sucrose (osmolarity 300 mOsm) saturated with 95%O_2–5%CO_2. 300-μM-thick horizontal dorsal hippocampal slices were cut and maintained in continuously oxygenated ACSF containing (in mM) 127 NaCl, 2 KCl, 1.5 $MgSO_4$, 25.7 $NaHCO_3$, 10 dextrose, and 1.5 $CaCl_2$ (pH 7.4; 300 mOsm). Whole-cell patch-clamp recordings were obtained from visually identified CA1 pyramidal neurons (CA1 neurons) at room temperature.

To record excitatory postsynaptic currents (EPSCs), patch electrodes (final resistance 3–6 MΩ) were filled with a filtered internal recording solution consisting of (in mM): 117.5 $CsMeSO_4$, 10 2-Hydroxyethyl] piperazine-N-[2-ethansulfonic acid] (HEPES), 0.3 N-[and glycol-bis (a-aminoethyl ether) N,N,N,N-tetraacetic acid (EGTA), 15.5 CsCl, and 1.0 $MgCl_2$, pH 7.3 (with CsOH); the osmolarity was 290–300 mOsm. Current-clamp recordings were performed using a recording solution containing (in mM) 100 potassium gluconate, 5 KCl, 10 HEPES, 1 EGTA, 2 $MgCl_2$, and 0.1 $CaCl_2$, pH 7.2 (with KOH). The electrode shank contained (in mM): 4 ATP Mg_2^+ salt, 0.3 GTP Na^+ salt and, for voltage clamp studies, 5 QX-314. Neurons were voltage clamped at −65 mV for the duration of the EPSC recordings. Whole-cell capacitance and series resistance (baseline 10–20 MΩ) were compensated by 80% at a 10 ms lag. Recordings were performed when the series resistance after compensation was less than 20 MΩ. The access resistance was monitored with a 10 ms, −5 mV test pulse and the recording was terminated if the series resistance increased by 25% any time during the experiment. Currents were filtered at 5 kHz, digitized using a Digidata 1322 digitizer (Molecular Devices, Sunnyvale, CA) and acquired using Clampex 8.2 software (Molecular Devices, Sunnyvale, CA).

Spontaneous EPSCs (sEPSCs) were recorded from CA1 neurons after blocking $GABA_A$ receptors with the antagonist picrotoxin (50 μM). Action-potential independent EPSCs (mEPSCs) were recorded by blocking action potentials with 1 μM tetrodotoxin (TTX, Alomone labs, Jerusalem, Israel). Evoked EPSCs (eEPSCs) were obtained by stimulating (2–8 V, 10 μs duration, and 0.07Hz) the Schaffer collateral pathway using a glass electrode filled with ACSF to evoke visually identifiable EPSCs. eEPSCs were analyzed only if they were not contaminated by spontaneous events. 30–50 eEPSCs were recorded at baseline and following drug application. They are individually analyzed using Clampfit 8.2 software (Molecular Devices, Sunnyvale, CA).

Current-clamp recordings were performed using the I = 0 (bridge mode) setting on the Axopatch 200B amplifier. The recordings were obtained only from cells whose baseline resting membrane potential (RMP) was −65 mV or lower. The firing rate of the neurons was studied using loose-patch recordings under conditions of elevated potassium (3 mM). The recording solution in these experiments was the same as that of the external perfusion solution.

The digitized current traces were analyzed with MiniAnalysis (Synaptosoft, Decatur, GA) as previously described (Kozhemyakin et al., 2010; Rajasekaran et al., 2007, 2009). The parameters used to detect EPSCs in the study were: Threshold = RMS * 3, period to search for local maximum 10,000 μs, time before peak for a baseline 80,000 μs, period to search for a decay 250,000 μs, fraction of peak to find decay time 0.001, and period to average a baseline 5000 μs. In sEPSC studies, a minimum of 150 events were detected at baseline and following drug application. For current-clamp recordings, the changes in RMP were determined using Clampfit 8.2 software (Molecular Devices, Sunnyvale, CA). For *in vitro* experiments, slices were obtained from at least 3–5 rats unless otherwise stated. The Kolmogorov–Smirnov (KS) test was used to compare cumulative distributions of frequency and amplitude of continuously recorded EPSCs. For the population, drug application data were analyzed using paired *t*-tests as these values represented repeated measures on the same neurons. Where more than 2 drugs were tested, a one way ANOVA with Tukey's post-hoc test was performed. Statistical analysis was performed using GraphPad Prism 5 software (GraphPad, Mountain View, CA). Data values are expressed as the mean ± SEM unless noted otherwise, and $p < 0.05$ was considered significant.

In vivo studies

Bipolar metal electrodes and an intraventricular cannula were implanted in the hippocampus and cortex as previously described (Martin and Kapur, 2008; Todorovic et al., 2012). Electrode headsets were connected to a Grass 7D amplifier and then to a Stellate digital system for electroencephalographic (EEG) recording.

SST, octreotide and lanreotide (Sigma, St. Louis, MO) were suspended in sterile saline and infused into the ventral hippocampus via a 28-gauge injection needle extending 1 mm past the cannula guide. The needle was connected to a 1.0 ml syringe, which was driven by an infusion pump (KD Scientific, Portland, OR) at a rate of 2.0 μl/min. Saline, SST, octreotide, or lanreotide were administered intracerebroventricularly (i.c.v.) at a rate of 120 μl/h for 3 h. The infusions were initiated 90 min before pilocarpine injection (50 mg/kg) or the beginning of hippocampal stimulation. EEG and video monitoring began 10 min prior to drug infusion and continued for up to 6 h after infusion was completed. Brief electrographic discharges (20–120 s durations) occurred during the initial infusion of saline or drugs and stopped prior to pilocarpine administration.

SE was induced by a combination of lithium–pilocarpine as previously described (Martin and Kapur, 2008). Animals were initially protected from the peripheral effects of cholinergic stimulation by the administration of scopolamine. However, in an initial study i.c.v. infusion of saline (70 μl) prevented SE in 57% (12/21) of the animals suggesting that the presence of the i.c.v. cannula sufficiently breached the blood brain barrier (BBB) to allow entry of scopolamine into the brain, preventing cholinergic-stimulation-induced SE. Scopolamine dose was subsequently eliminated, and all animals developed seizures and SE. In some animals, SE was induced by continuous hippocampal stimulation by stimulating the left ventral hippocampus with 10 s trains of 50 Hz, 1 ms, and 400 mA biphasic square wave current pulses