Somatostatin Presynaptically Inhibits Both GABA and Glutamate Release Onto Rat Basal Forebrain Cholinergic Neurons

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Submitted 16 May 2005; accepted in final form 24 March 2006

INTRODUCTION

The basal forebrain (BF) is a region in the forebrain that contains cholinergic and GABAergic corticopetal neurons in addition to various local circuit neurons (Zaborszky and Duque 2000, 2003). Loss of BF cholinergic neurons and concomitant deficits in cholinergic markers in the cortex constitute a hallmark of Alzheimer’s disease (AD) (Price et al. 1986). Studies combining EEG, juxtacellular labeling of recorded neurons with subsequent identification of their transmitter in anesthetized rats (Duque et al. 2000; Manns et al. 2000), or selective lesioning of the cholinergic neurons in combination with EEG monitoring during the sleep–wake cycle (Kapas et al. 1996) indicate that the generation of neocortical activation critically depends on cholinergic inputs from these regions.

Alterations in somatostatin (SS) levels and SS neuronal morphology have been observed in the cortex and BF of AD patients (Candy et al. 1983; Davies and Terry 1981; Francis et al. 1987; Kowall and Beal 1988; Roberts et al. 1985; Rossor et al. 1980). Behavioral experiments in rats suggests that mnemonic functions are impaired by depleting SS from central stores and this effect is mediated in part through the BF cholinergic system (Haroutunian et al. 1989). Intradiencephalic application or microinjection of SS-receptor agonists in forebrain areas result in sleep suppression (Obal and Krueger 2003).

Neurons expressing SS constitute a peptidergic interneuronal system in the septum, striatum, hippocampus, and cerebral cortex (Chesselet and Graybiel 1986; Forloni et al. 1990; Kohler and Eriksson; 1984; Vincent et al. 1985). In BF areas, patches of SS fibers and axons of local SS neurons were observed in close vicinity to cholinergic neurons (Zaborszky and Duque 2000), indicating a potential effect of SS on cholinergic neurons. Cholinergic neurons receive GABAergic input in BF areas (Zaborszky et al. 1986) and SS perikarya have been shown to be coexpressed with γ-aminobutyric acid (GABA) in many forebrain areas (Esclapez and Houser 1995; Hendry et al. 1984; Kosaka et al. 1988; Somogyi et al. 1984). A direct glutamate effect on cholinergic neurons is suggested by the presence of VGlut1- and VGlut2-type synapses on BF cholinergic neurons (Zaborszky et al. 2003). Although these morphological data raise the possibility of interactions among acetylcholine (ACh), SS, glutamate, and GABA, little information has been available regarding the functional role of SS in BF regions. Therefore using whole cell patch-clamp technique in forebrain slices of young rats, we investigated the effect of exogenously applied SS on GABAergic and glutamatergic transmission onto BF cholinergic neurons. Cholinergic neurons were identified by in vivo prelabeling with Cy3-192IgG, a selective marker of p75 neurotrophin receptor–expressing neurons (Wu et al. 2000). Preliminary data were previously published in abstract form (Momiyama and Zaborszky 2002, 2004).

METHODS

Labeling of BF cholinergic neurons with Cy3-192IgG for electrophysiology

All experiments were carried out in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiol-
logical Sciences of the Physiological Society of Japan (1998) and the UK Animals (Scientific Procedures) Act 1986. Young rats (10- to 14-days-old) were anesthetized with pentobarbital (50 mg/kg, administered intraperitoneally) and then mounted into a stereotaxic apparatus. Cy3-192IgG (3–4 μl; 0.4 mg/ml) was injected unilaterally into the lateral ventricle of each rat using a Hamilton syringe (22-gauge needle) at a rate of 0.5 μl/min (Wu et al. 2000). The coordinates of the lateral ventricle were 0.9 mm posterior from bregma, 1.1–1.2 mm lateral from midline, and 4 mm below from the dura.

Slice preparation for patch-clamp recordings

Three to six days after intracerebroventricular injection of Cy3-192IgG, rats were killed by decapitation under deep halothane anesthesia and coronal slices, containing the basal forebrain regions including the substantia innominata (SI) and the horizontal limb of the diagonal band (HDB), were cut (300 μm thick) using a microslicer (DTK-1000 or PRO7, Dosaka, Kyoto, Japan) in ice-cold oxygenated Krebs solution of the following composition (in mM): NaCl, 124; KCl, 3; CaCl2, 2.4; MgCl2, 1.2; NaH2PO4, 1; NaHCO3, 26; and d-glucose, 10; pH adjusted by 95% O2–5% CO2. The slices were then transferred to a holding chamber containing standard Krebs solution of the following composition (in mM): NaCl, 124; KCl, 3; CaCl2, 2.4; MgCl2, 1.2; NaH2PO4, 1; NaHCO3, 26; and d-glucose, 10; pH 7.4 when bubbled with 95% O2–5% CO2. Slices were incubated in the holding chamber at room temperature (21–26°C) for ≥1 h before recording.

Whole cell recording and data analysis

For recording, slices were transferred to the recording chamber, held submerged, and superfused with standard Krebs solution (bubbled with 95% O2–5% CO2) at a rate of 3–4 ml min⁻¹. Neurons in the SI or HDB were visually identified with a 60 × water-immersion objective attached to an upright microscope (BX50WI, Olympus Optics, Tokyo, Japan). Images were detected with a cooled CCD camera (CCD-300T-RC, Nippon Roper, Tokyo, Japan) and displayed on a video monitor (LC-150M1, Sharp, Osaka, Japan). Cy3-192IgG–labeled neurons were visualized using the appropriate fluorescence filter. Patch pipettes were made from standard-walled borosilicate glass capillaries (1.5 mm OD; Clark Electromedical, Reading, UK). For the recording of evoked or miniature synaptic currents, patch pipettes were filled with an internal solution of the following composition (in mM): CsCl, 140; NaCl, 9; Cs-EGTA, 1; Cs-HEPES, 10; Mg-ATP, 2 (pH adjusted with 1 M CsOH). Whole cell recordings were made from neurons labeled with Cy3-192IgG (Fig. 1, A and B) using a patch-clamp amplifier (Axopatch 200B, Axon Instruments, Foster City, CA). For the recording of the membrane properties and action potentials of the labeled neurons, another internal solution of the following composition (in mM) was used: KMeSO4, 135; KCl, 5; NaCl, 5; CaCl2, 0.1; K-EGTA, 1; K-HEPES, 5; Mg-ATP, 2; and Na5-GTP, 0.5 (pH adjusted with 1 M KOH). The cell capacitance and the series resistance were measured from the amplifier. The access resistance was monitored by measuring capacitative transients obtained in response to a hyperpolarizing voltage step (5 mV, 25 ms) from the holding potential of −65 mV. No correction was made for the liquid junction potentials (calculated to be 5.0 mV by pCLAMP7 software, Axon Instruments). Synaptic currents were evoked by delivering voltage pulses (0.2–0.4 ms in duration) of suprathreshold intensity at 0.2 Hz extracellularly by a stimulating electrode, made from the same glass capillary as for the recording pipette and filled with 1 M NaCl. The stimulating electrode was placed within a 50- to 120-μm radius of the recorded neuron. The position of the stimulating electrode was varied until a stable response was evoked in the recorded neuron. All the inhibitory and excitatory postsynaptic currents (IPSCs and EPSCs, respectively) were evoked at a holding potential of −65 mV. Experiments were carried out at room temperature (21–26°C).

Data were stored on digital-audio tapes using a DAT recorder (DC to 10 kHz; Sony, Tokyo, Japan). Evoked IPSCs and EPSCs were digitized off-line at 10 kHz (low-pass filtered at 2 kHz with an eight-pole Bessel filter) using pCLAMP8 software (Axon Instruments). The effects of SS or SS-receptor (sst) agonists on the evoked IPSCs or EPSCs was assessed by averaging the amplitude of IPSCs or EPSCs for 100 s (20 traces) during the peak response to each agonist and comparing this value with the averaged amplitude of 20 traces just before the agonist application. Miniature IPSCs and EPSCs (mIPSCs and mEPSCs, respectively) were filtered at 2 kHz and digitized at 20 kHz using pCLAMP8 software. The analyses of mIPSCs and mEPSCs were carried out using N software provided by Dr. S. F. Traynelis (Emory University). The effect of SS on mIPSCs or mEPSCs was assessed by comparing the frequency and amplitude distribution of the events for 5–10 min during the peak responses to SS with those obtained just before drug application. Statistical analysis was carried out using both Student’s t-test (two-tailed) and a nonparametric Mann–Whitney U test. The Kolmogorov–Smirnov (K-S) test was used for comparison of cumulative probability distribution of mIPSCs and mEPSCs. In all statistics, a P value of 0.05 was used as the confidence limit. Data are expressed as means ± SE.

Drugs

Cy3-192IgG was custom synthesized by Advanced Targeting Systems (San Diego, CA). Other drugs were stored in frozen stock solution and dissolved in the perfusing solution just before application.
in the final concentration indicated. All drugs were applied in the bath. Somatostatin was purchased from Peptide Institute (Osaka, Japan). 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX), d(-)-2-amino-5-phosphonopentanoic acid (d-AP5), octreotide, seglitide, and CYN 164806 were from Tocris Cookson (Bristol, UK). Bicuculline methochloride and strychnine were from Sigma (St. Louis, MO). Tetrodotoxin (TTX) was from Sankyo (Tokyo, Japan).

RESULTS

Cell identification

Whole cell recordings were made from a total of 94 Cy3-192IgG-stained neurons within the HDB or SI of the BF region. Figure 1, A and B shows a BF neuron under IR-DIC and stained with Cy3-192IgG. Membrane properties and action potential firing were examined in the current-clamp mode or voltage-clamp mode with KMeSO4-based internal solution in 28 of these 94 neurons. In current clamp, hyperpolarizing current injections produced inward rectification and depolarizing current injections produced outward rectification and depolarization in all of the 10 neurons tested (Fig. 1C). The inwardly rectifying currents were also observed in the voltage-clamp mode in all of the 10 neurons tested (Fig. 1D). Electrophysiological properties of all these 28 neurons (Fig. 1, C and D) agreed with those previously reported on cholinergic neurons within the BF region (Bengston and Osborne 2000; Wu et al. 2000). Therefore Cy3-192IgG–positive cells were identified as cholinergic neurons and used in the following experiments on SS-induced modulation of synaptic currents.

Effect of SS on the evoked IPSCs

After whole cell configuration was made from BF neurons stained with Cy3-192IgG, synaptic currents were evoked by focal stimulation in the presence of CNQX (5 μM), d-AP5 (25 μM), and strychnine (0.5 μM) to block non-NMDA (N-methyl-D-aspartate), NMDA, and glycine receptor components, respectively. The amplitude of the evoked synaptic currents was −369.9 ± 33.6 pA (n = 26). These synaptic currents were reversibly blocked by bath application of bicusculine (10 μM) in all of the four neurons tested (Fig. 2, A1 and A2), confirming that they were GABA A-receptor–mediated IPSCs. Bath application of somatostatin (SS, 1 μM) gradually reduced the amplitude of the evoked IPSCs, and the effect reached its steady state in several minutes (Fig. 2, B1 and B2). The IPSCs recovered to the control level after 5- to 10-min washout of SS (Fig. 2, B1 and B2). The magnitude of inhibition of the evoked IPSCs by SS (1 μM) was 34.4 ± 2.14% (n = 16, Fig. 7Ab). SS had no effect on the holding current with the present CsCl-based internal solution at the holding potential of −65 mV.

Effect of SS on the spontaneous mIPSCs

To dissect out whether the site of SS-induced action was presynaptic or postsynaptic, the effect of SS on the mIPSCs was examined in the presence of tetrodotoxin (TTX, 0.5 μM) in addition to CNQX (5 μM), d-AP5 (25 μM), and strychnine (0.5 μM). The frequency of mIPSCs was 0.65 ± 0.10 Hz (n = 15) in normal
Ca\textsuperscript{2+} (2.4 mM) external solution. Bath application of SS (1 μM) reduced the frequency of mIPSCs and the effect reached its steady state in 3–5 min (Fig. 3, A and B). The magnitude of inhibition of the mIPSC frequency by SS was 44.1 ± 3.72\% (n = 10) in normal (2.4 mM) Ca\textsuperscript{2+} concentration. On the other hand, SS had no significant effect on the amplitude distribution or mean amplitude of mIPSCs (Fig. 3, C and D). The relative amplitude of mIPSCs during application of SS was 102.3 ± 3.59\% (n = 10) of the control value in the 2.4 mM Ca\textsuperscript{2+}-containing solution.

Effect of SS on the evoked EPSCs

Synaptic currents were evoked in Cy3-192IgG–labeled BF neurons by focal electrical stimulation in the presence of bicuculline (10 μM), strychnine (0.5 μM), and d-AP5 (25 μM) to block GABA\textsubscript{A}–, glycine–, and NMDA-receptor–mediated current components, respectively. The amplitude of the evoked synaptic currents was −104.5 ± 11.2 pA (n = 16). As shown in Fig. 4, A1 and A2, these synaptic currents were reversibly blocked by bath application of CNQX (5 μM) in all of the four neurons tested, confirming that they were non-NMDA glutamatergic EPSCs. Similarly to the case of IPSCs, bath application of SS (1 μM) also inhibited the amplitude of evoked EPSCs in a reversible manner (Fig. 4, B1 and B2). The magnitude of inhibition of the evoked EPSCs by SS (1 μM) was 34.1 ± 2.62\% (n = 15, Fig. 7Bb).

Effect of SS on the spontaneous mEPSCs

The effect of SS on the mEPSCs was also examined in the presence of TTX (0.5 μM) in addition to bicuculline (10 μM), d-AP5 (25 μM), and strychnine (0.5 μM). The frequency of mEPSCs was 1.17 ± 0.23 Hz (n = 11) in normal Ca\textsuperscript{2+} (2.4 mM) external solution. Bath application of SS (1 μM) reduced the frequency of mEPSCs and the effect reached its steady state in 3–5 min (Fig. 5, A and B). The inhibition of the mEPSC frequency by SS was 50.1 ± 2.95\% (n = 11) in normal (2.4 mM) Ca\textsuperscript{2+} concentration. Similarly to the case of mIPSCs, SS had no significant effect on the amplitude distribution or mean amplitude of mIPSCs (Fig. 5, C and D). The relative amplitude of mIPSCs during application of SS was 97.8 ± 2.72\% (n = 11) of the control value, in 2.4 mM Ca\textsuperscript{2+}-containing solution.

Ca\textsuperscript{2+} dependency of SS-induced effects on mIPSCs and mEPSCs

The present results concerning SS-induced effects on mIPSCs and mEPSCs suggest that SS’s action is presynaptically mediated. To investigate whether the presynaptic action of SS is targeted at Ca\textsuperscript{2+} entry into the presynaptic terminal, we next examined the external Ca\textsuperscript{2+} concentration dependency of SS-induced effect on the mIPSCs and mEPSCs. After confirming SS-induced effect and recovery with washout in normal (2.4 mM) Ca\textsuperscript{2+}-containing solution, the external Ca\textsuperscript{2+} concentration was raised to 7.2 mM in the same neuron and SS was applied again. Furthermore, in this series of experiments, sucrose was added in the normal solution to make it isotonic with the high-Ca\textsuperscript{2+} solution. The frequency of mIPSCs increased from 0.68 ± 0.13 Hz (n = 6) to 1.61 ± 0.46 Hz (n = 6) when external Ca\textsuperscript{2+} concentration was raised to 7.2 mM. In the solution containing 7.2 mM Ca\textsuperscript{2+}, SS inhibited the frequency of mIPSCs by 74.6 ± 3.32\% (n = 6) of control, significantly (P < 0.05) larger than that in normal Ca\textsuperscript{2+} concentration (44.7 ± 2.54\%, n = 6, Fig. 6A). The relative amplitude of mIPSCs during application of SS was 100.8 ± 4.04\% (n = 6), and 101.9 ± 1.91\% (n = 6) of the respective control value, in 2.4 and 7.2 mM Ca\textsuperscript{2+}-containing solution, respectively.

Similarly to the case of mIPSCs, the frequency of mEPSCs increased from 0.99 ± 0.17 Hz (n = 6) in normal Ca\textsuperscript{2+} (2.4 mM) solution to 1.95 ± 0.73 Hz (n = 6) in the high-Ca\textsuperscript{2+} (7.2 mM) external solution. The inhibition of the mEPSC frequency by SS was 47.6 ± 1.87\% (n = 6) of control, significantly (P < 0.05) larger than that in normal Ca\textsuperscript{2+} concentration (24.7 ± 2.81\%, n = 6, Fig. 6B). SS-induced inhibition of mEPSC frequency was significantly (P < 0.05) larger in 7.2 mM Ca\textsuperscript{2+}-containing solution than in normal Ca\textsuperscript{2+} concentration (Fig. 6B).
relative amplitude of mEPSCs during application of SS was 99.1 ± 4.68% (n = 6), and 101.7 ± 2.33% (n = 6) of the respective control value, in 2.4 and 7.2 mM Ca^{2+}-containing solution, respectively.

Pharmacological identification of SS-receptor subtypes

Although there is a lack of highly selective SS-receptor–related drugs, some drugs are available for elucidating SS-
of IPSCs and EPSCs. Octreotide is not expected to mimic the actions of SS if they are mediated by sst1 or sst4 subtypes, whereas seglitide will not mimic sst1-mediated actions (Hannon et al. 2002; Raynor et al. 1993). CYN154806 should block only sst2 subtype (Hannon et al. 2002; Mastrodimou et al. 2006; Nunn et al. 2002).

**IPSCs.** First, the effects of octreotide and seglitide on the amplitude of IPSCs were compared with those on SS (Fig. 7A). Octreotide at a concentration of 1 μM inhibited IPSCs by 29.2 ± 5.11% (n = 6, Fig. 7A). Seglitide (1 μM) also inhibited the IPSCs by 30.2 ± 10.1% (n = 5, Fig. 7A). The effect of octreotide or seglitide was not significantly (P > 0.05) different from that of SS (34.4 ± 2.14%, n = 16, Fig. 7A). These results suggest that SS-induced action on the IPSCs is not mediated by sst1 or sst4 subtypes. To further examine this possibility, the effect of CYN 154806 (1 μM) on SS (1 μM)-induced inhibition of IPSCs was examined (Fig. 8A). After confirming the effect of SS and recovery on washout, the antagonist was applied for 10 min, and then SS was applied again in the presence of the antagonist. Application of CYN 154806 itself had no effect on the IPSCs in any of six neurons tested. In the presence of CYN 154806, SS-induced inhibitory effect on the IPSCs was reduced to 11.2 ± 1.21% (n = 6, Fig. 8A), which was significantly (P < 0.05) smaller than that of SS alone obtained in the corresponding six neurons (39.4 ± 3.64%, Fig. 8A). These results suggest that SS-induced action of the IPSCs is mediated mostly by the sst2 subtype.

**EPSCs.** Similarly to IPSCs, the effects of octreotide and seglitide on the amplitude of EPSCs were examined. Unlike the case of IPSCs, octreotide- or seglitide-induced inhibitory effect was not prominent. The inhibition of EPSCs by octreotide (1 μM) or seglitide (1 μM) was 8.12 ± 5.11% (n = 6) or 8.83 ± 2.92% (n = 6), respectively, which was significantly (P < 0.05) smaller than that by SS (1 μM, 34.1 ± 2.62%, n = 15, Fig. 7B). Furthermore, even in the presence of CYN 154806 (1 μM), SS still inhibited the EPSCs by 32.9 ± 4.66% (n = 6, Fig. 8B), which was not significantly (P > 0.05) different from the value with SS alone in the corresponding six neurons.

**Fig. 6.** Ca²⁺ dependency of SS-induced effect on the frequency of mIPSCs and mEPSCs. Histograms summarizing the inhibitory effect of SS on the frequency of mIPSCs (A) and mEPSCs (B) in 2.4 and 7.2 mM Ca²⁺. A: values for 2.4 and 7.2 mM Ca²⁺ were 44.7 ± 2.54% (n = 6) and 74.6 ± 3.32% (n = 6), respectively. Effect in 7.2 mM Ca²⁺ (*) was significantly (P < 0.05) larger than that in 2.4 mM Ca²⁺. B: effect in 7.2 mM Ca²⁺ (69.7 ± 2.81%, n = 6, *) was significantly (P < 0.05) larger than that in 2.4 mM Ca²⁺ (47.6 ± 1.87%, n = 6).

**Fig. 7.** Effects of octreotide (OCT) and seglitide (SEG) on the IPSCs and EPSCs. All IPSCs or EPSCs were evoked at 0.2 Hz at the holding potential of −65 mV. A: inhibitory effects of OCT or SEG on the IPSCs. Each trace in Aa and Ab is the superimposed averages of 20 consecutive synaptic currents before (control) and during agonist application (OCT or SEG). Ab and Bb: histograms summarizing the mean inhibitory effects of OCT, SEG, and somatostatin (SS) on the IPSCs (Ab) and EPSCs (Bb). Error bars indicate SE. Concentration of each agonist was 1 μM. Ab: values for OCT and SEG were 29.2 ± 5.11% (n = 5) and 30.2 ± 10.1% (n = 5), respectively, which were not significantly (P > 0.05) different from that of SS (34.4 ± 2.14%, n = 16), Bb: values for OCT and SEG were 8.12 ± 5.11% (n = 6) and 8.83 ± 2.92% (n = 6), respectively, which were significantly (*P < 0.05) smaller than that of SS (34.1 ± 2.62%, n = 15).

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(34.0 ± 2.76%, n = 6, Fig. 8B). These results suggest that sst receptor subtypes mediating SS-induced inhibition of EPSCs are mainly sst1 or sst4.

DISCUSSION

The present findings demonstrate that exogenously applied SS presynaptically inhibits both GABA and glutamate release onto BF cholinergic neurons. They also suggest that SS-induced inhibition of GABA and glutamate release is mediated by different subtypes of sst receptors. The present results on the mIPSCs and mEPSCs suggest that SS action on the transmission is mediated in a calcium-dependent way. It remains to be elucidated whether activation of presynaptic sst receptors actually leads to blockade certain types of calcium channels. Although presynaptic modulation by SS has been reported in several central excitatory or inhibitory synapses, including hippocampus (Boehm and Betz 1997; Tallent and Siggins 1997), sensory thalamus (Leresche et al. 2000), hypothalamus (Lanneau et al. 2000), and the reticular thalamic nucleus (Sun et al. 2002), a potential role for SS in the modulation of BF synaptic transmission has been demonstrated for the first time in the present study. Indeed, the modulatory role of SS for inhibiting both GABA and glutamate release onto the BF cholinergic neurons should be unique.

**GABAergic and glutamatergic afferents to BF cholinergic neurons**

Cholinergic neurons in the ventral pallidum and substantia innominata receive heavy GABA input (Zaborszky et al. 1984); and amygdaloid lesions have been found to result in decreased glutamate uptake in the substantia innominata (Francis et al. 1987). In addition, it has been suggested that putative glutamatergic afferents from the mesopontine tegmentum could affect cortical ACh release by basal forebrain cholinergic neurons (Rasmussen et al. 1994), although no direct morphological evidence supports this notion. Furthermore, glutamatergic afferents to BF cholinergic neurons may originate from local Vglut2 neurons (Hur and Zaborszky 2005).

**Mechanism of SS action on GABA and glutamate release**

The present electrophysiological results show that exogenously applied SS presynaptically inhibits both GABA and glutamate release onto cholinergic neurons in the BF. Electrophysiological properties of the BF neurons identified by Cy3-192IgG well agreed with those reported previously (Bengtson et al. 1987). In addition, it has been suggested that putative glutamatergic afferents from the mesopontine tegmentum could affect cortical ACh release by basal forebrain cholinergic neurons (Rasmussen et al. 1994), although no direct morphological evidence supports this notion. Furthermore, glutamatergic afferents to BF cholinergic neurons may originate from local Vglut2 neurons (Hur and Zaborszky 2005).
neurons in these BF regions. The present pharmacological analyses using sst-receptor–specific drugs could provide more detailed insights that SS-induced inhibition of GABA and glutamate release is mediated by different sst subtypes; SS-induced inhibition of GABA release is mediated by mostly sst2 receptors, whereas the inhibition of glutamate release is mainly mediated by sst1 or sst4 receptors. The present finding of sst2-receptor–mediated inhibition of GABA release well agrees with a recent study by Bassant et al. (2005). The work has demonstrated that GABAergic neurons in the medial septum/diagonal band of Broca express sst2A receptors, activation of which by octreotide inhibits the activities of the GABAergic neurons. The present data suggest that SS has a role in modulating the excitability of BF cholinergic neurons by inhibiting both inhibitory and excitatory transmissions, as reported in the case of dopamine (Momiyama and Sim 1996; Momiyama et al. 1996). Thus it is likely that SS acts to balance between excitatory and inhibitory inputs to BF cholinergic neurons. Although no postsynaptic effects were observed in the present electrophysiological studies using CsCl-based internal solution, we have observed in our preliminary experiments using K-based internal solution that SS (1 µM) hyperpolarized the membrane of putative cholinergic neurons in the BF in a similar time course to that required to reduce transmitter release (unpublished observations). Detailed mechanisms underlying postsynaptic effects of SS remain to be elucidated to construct a complete picture of SS’s role in controlling the excitability of BF cholinergic neurons.

At present the mechanism of how endogenous SS release can influence GABA or glutamate release onto cholinergic neurons is unclear. Actually, the present study does not show that synaptically released SS can affect transmission. Thus it seems unlikely that synaptically released SS would inhibit excitatory and inhibitory transmission onto the same neuron to the same extent. With respect to the functional interpretation of SS action on cholinergic neurons, it is important to know whether the SS terminals on cholinergic neurons coexpress GABA. The most detailed study of GABA and SS coexistence to date has been done in the hippocampus (Esclapez and Houser 1995; Kosaka et al. 1988). According to these studies, 30–50% of dentate hilar GABA neurons contain SS, whereas the rest of GABA neurons contain other peptides.

Behavioral significance of SS/cholinergic interaction in the basal forebrain

Several behavioral studies suggest the role of SS in cognitive processes (DeNoble et al. 1989; Haroutunian et al. 1987; Malthe-Sorensen et al. 1978; Sunderland et al. 1987; Vecsei et al. 1984) or control of sleep conditions (Beranek et al. 1997; Hajdu et al. 2002). In addition the recent study mentioned above (Basant et al. 2005) showed that intraseptal injections of octreotide or SS in freely moving rats reduce the power of hippocampal EEG in the theta band by activating sst2A receptors, suggesting a mechanism in the control of theta activity. It might be possible that the effect of SS on sleep conditions is mediated at least partly by modulating the GABAAergic/cholinergic interaction in the BF (Zaborszky et al. 1986).

For a comprehensive understanding of the behavioral relevance of GABA–SS–AOh–glutamate interactions in the BF, combined morphological–electrophysiological in vitro and in vivo studies are necessary, which 1) identify the origin of SS input to BF cholinergic neurons, 2) determine the cellular and subcellular localization of SS receptors, 3) correlate the firing properties of SS and cholinergic neurons as they relate to EEG epochs along the entire sleep–wake cycle, and 4) establish the precise local synaptic circuitry of the BF.

Acknowledgments

We are grateful to F. Nagy for constructive comments on the manuscript.

Grants

This work was supported by Grants-in-Aid (13680904 and 15016106) for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, Core Research for Evolutional Science and Technology of Japan Science and Technology Corporation, Astra Zeneca Research Grants 2001 and 2003 to T. Momoyama, and U.S. Public Health Service Grant NS-23945 to L. Zaborszky.

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