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Laminar and cellular analyses of reduced somatostatin gene expression in the subgenual anterior cingulate cortex in major depression

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ABSTRACT

Somatostatin (SST), a neuropeptide expressed in dendritic-targeting gamma-aminobutyric acid (GABA) neurons, is decreased across corticolimbic areas in major depressive disorder (MDD). SST-positive GABA neurons form heterogeneous subgroups with different laminar distributions and electrophysiological properties, so knowing the anatomical and cellular localization of reduced SST may provide insight into the nature of the pathology in MDD. In cohorts of MDD subjects with known reduction of SST in postmortem sgACC gray matter, we used in situ hybridization to quantify the laminar and cellular patterns of altered SST mRNA expression. SST mRNA levels were lower across all cortical layers in the MDD subjects. Expression levels per cell were also lower, but the density of labeled neurons did not differ between subject groups. Consistent with the previous tissue level analysis, differences were more robust in females. In summary, we report MDD-related reduction in SST expression per cell across cortical layers in sgACC, suggesting a general vulnerability of SST neurons independent of specific cell type.

Introduction

Somatostatin (SST), a neuromodulatory peptide, is expressed in a subtype of GABA neurons that inhibits the dendritic compartment of principal excitatory glutamatergic neurons (Viollet et al., 2008). Lower SST expression has been reported in psychiatric and neurodegenerative disorders, including schizophrenia (Morris et al., 2008), bipolar disorder (Konradi et al., 2011) and Alzheimer's disease (Gahete et al., 2010). In major depressive disorder (MDD), we reported a downregulation of SST mRNA expression in the dorsolateral prefrontal cortex (Sibille et al., 2011), subgenual anterior cingulate cortex (sgACC) (Tripp et al., 2011, 2012) and in the lateral and basomedial nuclei of the amygdala (Guilloux et al., 2012) compared to that in control subjects. Differences were more robust in female MDD subjects in sgACC and were restricted to women with MDD in the amygdala (Guilloux et al., 2012; Sibille et al., 2009). These findings were consistent with postmortem studies showing reduced calbindin-positive GABA neuron density in MDD (Maciag et al., 2010; Rajkowska et al., 2007), as SST is largely co-localized with calbindin [reviewed in (Viollet et al., 2008)].

SST cells represent ~18–20% of interneurons across cortical regions, but are not evenly distributed across cortical layers (Lewis et al., 1986; McDonald and Mascagni, 2002; Weckbecker et al., 2003). At least three different subtypes of SST neurons have been identified through the generation of transgenic mice expressing green fluorescent protein (GFP) in SST cells (Ma et al., 2006; Oliva et al., 2000). So-called GIN and X98 mice express GFP in layers 2/3 and 5 SST neurons, and send abundant projections to layer 1. These neurons include the traditional low threshold spiking Martinotti cells that provide inhibitory inputs to the distal dendrites of pyramidal neurons. In contrast, GFP-expressing cells in X94 mice represent a population of SST neurons in layer 4 (or deep layer 3 in agranular sgACC), which do not project to layer 1, display "stuttering" electrophysiological properties, and target layer 4 parvalbumin-positive GABA neurons (Xu et al., 2013). Consistent with these differences in innervation patterns, optogenetic-induced activity of layer 2/3/5 SST neurons resulted in pyramidal cell inhibition, while activity of layer 4 (or deep layer 3 in agranular sgACC) resulted in pyramidal cell disinhibition (Xu et al., 2013). Accordingly, in MDD, a reduction in SST function in agranular layer 3 of the sgACC might result in decreased excitation of pyramidal cells. On the other hand, reduction in SST function in layers 2/3/5 might result in increased excitation of pyramidal cells.

Accordingly, understanding whether all or a specific subset of SST cells are affected in MDD has important implications for understanding the nature of the circuit dysfunction. Here, we tested two alternative hypotheses regarding the role of SST reduction in MDD. First, SST

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reduction seen in MDD represents shared biological vulnerability of SST cells in general. Based on this hypothesis, we would predict similar changes in SST cells across all layers. Second, SST reduction represents secondary, adaptive changes in the brain due to a putative primary deficit in pyramidal cell function. Based on this alternative hypothesis, we would predict different results in sgACC deep layer 3 versus layers 2/3 and 5, based on their opposite effects on pyramidal cells. To test these hypotheses, we used in situ hybridization to quantify the laminar and cellular patterns of altered SST mRNA expression in the sgACC of MDD subjects. Note that these studies were performed in the same subjects for which we had a priori information on reduced SST expression, as measured by gene array and quantitative PCR in combined gray matter samples (Tripp et al., 2011), so it is not a replication of those initial findings, but rather a follow-up on reduced SST expression at the cortical layer and cell level.

Materials and methods

Human postmortem subjects

Brain specimens were obtained during routine autopsies conducted at the Allegheny County, PA, Medical Examiner's Office after consent was obtained from next of kin. An independent committee of experienced research clinicians made consensus DSM-IV diagnoses for each subject using structured interviews with family members and review of medical records. The absence of psychiatric diagnoses was confirmed in comparison subjects through the same approach. To control for biological variance, subjects with MDD were matched to comparison subjects for sex and as closely as possible for age (Table 1). There were no statistical differences between MDD and control groups on any demographic or technical parameters. Subjects were selected from a previous study (Tripp et al., 2011) so as to enrich our cohort in MDD subjects with robust SST expression reduction, with the goal of characterizing the layer and cellular specificity of the previously reported SST reduction. Twenty MDD and nineteen control subjects (~50% female) were investigated. All procedures were approved by the University of Pittsburgh Committee for the Oversight of Research Involving the Dead and Institutional Review Board for Biomedical Research.

In situ hybridization

In situ hybridization probes, methods and analytical protocols were as previously described in (Morris et al., 2008). The templates for the synthesis of the antisense and sense riboprobes were generated by PCR. The specific primers amplified a 337 base pair fragment, corresponding to bases 112–448 of the human SST gene (GenBank NM_001048). This SST riboprobe was used and described previously (Guilloux et al., 2012; Morris et al., 2008). ^{35}S-labeled riboprobes were generated by in vitro transcription, and hybridized to three sections from each subject; the three sections were separated by 200 μm. MDD and control subjects were processed in parallel to reduce experimental variance. Each section was randomly coded, so that subject number and diagnosis were unknown to the experimenter. Glass slide sections were trans-illuminated and brightfield images were captured on video camera, digitized, and recorded. Areas were defined on brightfield digitized images of the dried in situ hybridization slides. After digitizing each autoradiographic film section, the slides were coated with liquid emulsion at 43°C. (KODAK Autoradiography Emulsion, Type NTB for light Microscope Autoradiography Rochester, NY). Slides were dipped for 1 min using an automated dipping machine. Slides were wrapped in aluminum foil in a plastic box and incubated at 4°C. After 12 days, the coated slides were dipped for 4 min in developer, 1 min in water and 8 min in fixer. After the emulsion coated slides were developed, they were immediately stained for 10 s with 0.5% cresyl violet solution and washed two times in Milli-Q water. We used published measures (Gittins and Harrison, 2004) to delineate each cortical layer on these cresyl violet stained sections.

Optical density of SST mRNA expression by layer

Optical density was measured using film autoradiographs. Using the Microcomputer Imaging Device (MCID; Imaging Research Inc., London,

Table 1
Demographic and postmortem characteristics of human subjects for in situ hybridization study.

Comparison subjects								MDD subjects								
Case	Sex	Race	Age	PMI	pH	RIN	Cause of death	Case	Sex	Race	Age	PMI	pH	RIN	Suicide	Cause of death	Medications ATOD
546	F	W	37	23.5	6.7	8.6	ASCVD	666	F	W	16	10	7.3	9.2	N	Trauma	D
818	F	W	67	24	7.1	8.4	Anaphylactic reaction	934	F	W	54	17.9	6.2	8.2	N	ASCVD	D O
1081	F	W	57	14.9	6.8	9	COPD	1041	F	W	52	10.3	6.5	8.4	N	Drug overdose	B D O P
1092	F	B	40	16.6	6.8	8	Mitral valve prolapse	1221	F	B	28	24.8	6.6	7.2	N	Pulmonary thrombosis	N
1099	F	W	24	9.1	6.5	8.6	Cardiomyopathy	1249	F	W	40	11.2	6.5	9	N	Drug overdose	B C D O
1247	F	W	58	22.7	6.4	8.4	ASCVD	1254	F	W	39	12.8	6.4	9	Y	Incised wounds	D
1280	F	W	50	23.5	6.7	7.7	Pulmonary thromboemboli	1289	F	W	46	25	6.3	7.3	N	ASCVD	U
1391	F	W	51	7.8	6.6	7.1	ASCVD	1315	F	W	28	12.4	7	7.9	Y	Asphyxiation by hanging	N
1403	F	W	45	12.3	6.7	8.2	ASCVD	1356	F	W	60	20.6	6.1	8.5	N	Intraperitoneal hemorrhage	D O
10013	F	W	16	9.3	6.7	9	Trauma	1360	F	W	59	18.1	6.4	7.6	Y	Drowning	D
615	M	W	62	7.2	6.4	7.8	Ruptured aortic aneurysm	513	M	W	24	13.1	6.9	7.0	Y	Asphyxiation by hanging	N
634	M	W	52	16	7	8.1	ASCVD	600	M	W	63	9.9	6.7	7.1	Y	Asphyxiation by hanging	O
789	M	W	22	20	7	7.4	Asphyxiation	619	M	W	55	18.8	6.9	7.9	Y	GSW of head	B D
852	M	W	54	8	6.8	9.1	Cardiac tamponade	809	M	W	50	20	6.9	8.5	N	ASCVD	D O
1031	M	W	53	23.1	6.8	8.2	Arteriosclerotic/ hypertensive CVD	863	M	W	51	28.3	7.2	8.4	N	ASCVD	N
1047	M	W	43	12	6.9	9	ASCVD	943	M	W	56	15.4	6.6	8.2	Y	GSW to mouth	O
1086	M	W	51	24.2	6.8	8.1	ASCVD	1001	M	W	53	7.3	6.6	7.6	N	Arteriosclerotic/ hypertensive heart disease	O
1317	M	W	56	22.9	6.4	6.9	ASCVD	1312	M	W	51	24.6	6.5	8.1	N	Drug overdose	O
1372	M	W	37	20.5	6.6	9	Accidental asphyxiation	1320	M	W	55	24.4	6.5	7.2	N	ASCVD	N
								10,031	M	W	36	20	6.8	8.9	N	Drug overdose	C D P
Mean			46.1	16.7	6.7	8.2					45.8	17.2	6.6	8.1			
SD			13.8	6.4	0.2	0.7					13.3	6.2	0.3	0.7			

Abbreviations: ASCVD, arteriosclerotic cardiovascular disease; ATOD, at time of death; B in Medications ATOD column, benzodiazepines; B in race column, black subject; C, anticonvulsants; COPD, chronic obstructive pulmonary disease; D, antidepressants; F, female; GSW, gunshot wound; M, male; MDD, major depressive disorder; N, no medications; O, other medication(s); P, antipsychotics; PMI, postmortem interval in hours; RIN, RNA integrity number; W, white subject.