Neuroscience 346 (2017) 320–336

HIGHER SUSCEPTIBILITY OF SOMATOSTATIN 4 RECEPTOR GENE-DELETED MICE TO CHRONIC STRESS-INDUCED BEHAVIORAL AND NEUROENDOCRINE ALTERATIONS

BÁLINT SCHEICH, [a,b,d]* **KATA CSEKő,** [a,b,d] **ÉVA BORBÉLY,** [a,b,d] **ISTVÁN ÁBRAHÁM,** [b,d,e,g] **VALÉR CSERNUS,** [c] **BALÁZS GASZNER** [c,d] **AND ZSUZSANNA HELYES** [a,b,d,f]

[a] *Department of Pharmacology and Pharmacotherapy, University of Pécs Medical School, Pécs, Hungary*

[b] *János Szentágothai Research Center, University of Pécs, Pécs, Hungary*

[c] *Department of Anatomy, University of Pécs Medical School, Pécs, Hungary*

[d] *Center for Neuroscience, University of Pécs Medical School, Pécs, Hungary*

[e] *Institute of Physiology, University of Pécs Medical School, Pécs, Hungary*

[f] *MTA-PTE NAP B Chronic Pain Research Group, Hungary*

[g] *MTA-PTE NAP B Molecular Neuroendocrinology Research Group, Hungary*

Abstract—The somatostatin 4 receptor (sst_4) is widely expressed in stress-related brain areas (e.g. hippocampus, amygdala) and regulates the emotional behavior in acute situations. Since its importance in chronic stress-induced complex pathophysiological alterations is unknown, we investigated the involvement of sst_4 in the responsiveness to chronic variable stress (CVS). *Sstr4* gene-deficient (*Sstr4*$^{-/-}$) mice and their wildtype counterparts (*Sstr4*$^{+/+}$) were used to examine the behavioral and neuroendocrine alterations as well as chronic neuronal activity (FosB expression) changes in response to CVS. In *Sstr4*$^{+/+}$ mice, there was no behavioral response to the applied CVS paradigm. In contrast, immobility time in the tail suspension test increased after the CVS in the knockouts. In the forced swim test, *Sstr4*$^{-/-}$ animals showed increased baseline immobility and then it decreased after the CVS. Light–dark box and open field test behaviors and sucrose preference did not respond to the stress in the knockouts. Adrenal weights increased and thymus weights decreased in both *Sstr4*$^{+/+}$ and *Sstr4*$^{-/-}$ mice demonstrating the effect of chronic stress. The relative adrenal weight of stressed knockouts increased to a greater extent, while relative thymus and body weights decreased only in the *Sstr4*$^{-/-}$ mice. Basal plasma corticosterone concentrations did not change after the CVS in either genotype. FosB immunopositivity in the central and basolateral amygdaloid nuclei was enhanced in stressed knockouts, but not in wild types. This is the first evidence that sst_4 activation is involved in the behavioral and neuroendocrine alterations induced by chronic stress with a crucial role of plastic changes in the amygdala.

Key words: somatostatin, somatostatin 4 receptor, depression, chronic variable stress, FosB.

INTRODUCTION

The inhibitory neuropeptide somatostatin is expressed in several cerebral regions and plays important roles in physiological and pathological brain functions including mood disorders (Martel et al., 2012). Clinical studies revealed that the level of somatostatin is reduced in the dorsolateral prefrontal (Sibille et al., 2011) and cingulate cortex (Tripp et al., 2011) and in the amygdala (Guilloux et al., 2012) of patients with major depressive disorder. A decrease in somatostatin expression has also been found in chronically stressed rats, an experimental model of depression (Czéh et al., 2015). In these studies, somatostatin was used as a marker of a subpopulation of GABAergic interneurons, but the importance of the peptide as a neuromodulator has also been proven by recent experimental findings. Somatostatin is released in the amygdala (Brodin et al., 1994), hippocampus (Arancibia et al., 2001) and hypothalamus (Arancibia et al., 2000) in response to acute stress. Mice lacking the gene of somatostatin show behavioral, endocrine and neurobiological changes resembling to those seen in depressed patients (Lin and Sibille, 2015). The peptide exerts anxiolytic and antidepressant-like effects after both

*Correspondence to: B. Scheich, Department of Pharmacology and Pharmacotherapy, University of Pécs Medical School, Szigeti Street 12, H-7624 Pécs, Hungary. Fax: +36-72-536-218.
E-mail addresses: scheich.balint@gmail.com (B. Scheich), csekoe.kata@gmail.com (K. Csekő), eva.borbely@aok.pte.hu (É. Borbély), istvan.abraham@aok.pte.hu (I. Ábrahám), valer.csernus@aok.pte.hu (V. Csernus), balazs.b.gaszner@aok.pte.hu (B. Gaszner), zsuzsanna.helyes@aok.pte.hu (Z. Helyes).

Abbreviations: CVS, chronic variable stress; OFT, open field test; SPT, sucrose preference test; LDB, lightdark box test; TST, tail suspension test; FST, forced swim test; HPA-axis, hypothalamo-pituitary-adrenal axis; CeA, BLA and MeA, central, basolateral and medial amygdaloid nuclei, respectively; ovBST, dlBST, dmBST, vBST, oval, dorsolateral, dorsomedial and ventral nuclei of the bed nucleus of the stria terminalis, respectively; DG, dentate gyrus; vLS and dLS, ventral and dorsal lateral septum, respectively; pPVN and mPVN, parvocellular and magnocellular parts of the paraventricular nucleus of the hypothalamus, respectively; dRN, dorsal raphe nucleus; EWcp, central projecting Edinger–Westphal nucleus; dPAG and lPAG, dorsal and lateral parts of the mesencephalic periaqueductal gray matter, respectively.

http://dx.doi.org/10.1016/j.neuroscience.2017.01.039
0306-4522/

intracerebroventricular (Engin et al., 2008) and intra-amygdalar or intra-septal (Yeung et al., 2011) injection in rat models. The importance of somatostatin in the antidepressant action of imipramine has also been raised (Nilsson et al., 2012).

Despite the role of somatostatin in emotional regulation, the relevance of its five G-protein-coupled receptors is much less known. Anxiolytic and antidepressant-like effects of sst_2 and sst_3 stimulation have been shown by studies with acute agonist (Engin and Treit, 2009) and antagonist (Yeung and Treit, 2012) treatments in rats. Elevated anxiety measures accompanied by higher pituitary corticotropin in mice lacking the *Sstr2* gene (Viollet et al., 2000) and a selective increase in sst_2 mRNA expression in the amygdala and anterior cingulate cortex after acute stress (Nanda et al., 2008) were also described. Regulatory role of the sst_4 in the behavior and neuronal activation of stress-related brain areas were revealed in acute stressful situations by the recent data of our group with *Sstr4* gene-deleted mice and a selective sst_4 agonist (Scheich et al., 2016). These studies were inspired by previous data showing the expression of sst_4 in rodent and human stress-related brain regions (Schreff et al., 2000; Selmer et al., 2000a,b). Although these experimental results from acute models provide valuable information about the involvement of somatostatin and its receptors in the emotional regulation, chronic stress models have much more translational relevance in relation to human mood disorders.

Since chronic stress is a well-known etiological factor in depression, several rodent models have been introduced to reveal its exact underlying mechanisms and mediators. Among these, chronic variable stress (CVS) originally described by Katz and colleagues (Katz and Hersh, 1981; Katz et al., 1981) has been shown to be a very reliable, valid and translationally relevant experimental paradigm. Several functional alterations being parallel to the symptoms of human depression have been found in animals exposed to CVS, including anhedonia, anxiety, depression-like behavior and neuroendocrine changes, such as increased activity of the hypothalamo-pituitary-adrenal (HPA) axis (Willner, 1997). Additionally, several studies found neurobiological alterations being similar to those found in patients with depression (Hill et al., 2012).

Based on our recent findings providing evidence for the role of sst_4 in emotional control, we aimed at analyzing the relevance of this receptor in the responsiveness to CVS in a mouse model. To address this issue, *Sstr4* gene-deleted mice ($Sstr4^{-/-}$) and their wild-type siblings ($Sstr4^{+/+}$) were exposed to a CVS procedure. We used a relatively short, 3-week-long CVS paradigm, which does not result in robust changes in wild-type mice, in order to reveal potentially more apparent functional alterations in the knockouts showing their increased susceptibility to this "mild" chronic stress. Stress-sensitivity was examined by assessing behavioral and endocrine alterations and changes of the neuronal activity in stress-related neuronal circuits in the brain.

EXPERIMENTAL PROCEDURES

Animals

We used 8-week-old *Sstr4* gene-deleted ($Sstr4^{-/-}$) mice and their wild-type ($Sstr4^{+/+}$) siblings ($N = 39$, 9–11/group) generated on C57Bl/6 background divided into 4 groups: control and chronically stressed wild types and knockouts. Only male mice were included in order to avoid the confounding effect of the estrous cycle (Carey et al., 1995). The original heterozygous ($Sstr4^{+/-}$) breeding pairs were produced and kindly donated by Prof. Piers C Emson (Laboratory of Molecular Neuroscience, The Babraham Institute, Babraham Research Campus, Babraham, Cambridge CB22 3AT, United Kingdom). *Sstr4* gene-deleted mice were generated using a LacZ-containing construct (Helyes et al., 2009). The $Sstr4^{+/+}$ and $Sstr4^{-/-}$ mice were the offspring of heterozygous animals originating from the 10th generations of backcrossed mice (using $Sstr4^{+/-}$ and C57Bl/6, males and females alternately). The genotypes of $Sstr4^{+/+}$, $Sstr4^{+/-}$ and $Sstr4^{-/-}$ mice were determined with PCR. Briefly, approximately 1–2 mm from the end of their tail was cut 4 weeks after birth. DNA was purified and amplified by Phire Tissue Direct PCR Kit (Thermo Scientific) using an Eppendorf Mastercycler gradient. The following three primers were used in the reaction mix: LacZ, Sstr4 769 and Sstr4 1336. The amplified DNA was detected with 2% agarose gel electrophoresis. The $Sstr4^{+/+}$ band was 568 kb, the $Sstr4^{-/-}$ band was 500 kb and both were detected in case of $Sstr4^{+/-}$ mice.

All animals were kept in the Laboratory Animal House of the Department of Pharmacology and Pharmacotherapy of the University of Pécs Medical School, housed in standard plastic cages (4–6 mice/cage) under standard conditions 24 and 25 °C, 12–12-h light–dark cycle) and provided with standard rodent chow and water *ad libitum*.

The experimental design was in accordance with the recommendations of the 1998/XXVIII Act of the Hungarian Parliament on Animal Protection (243/1988) and were approved by the Ethics Committee on Animal Research of University of Pécs (license No. BA02/2000-25/2011). We made all efforts to minimize the number and suffering of the animals used in this study.

Experimental procedure and CVS paradigm

The whole experiment lasted for 4 weeks. In the first week, we performed control functional measurements (1st trial) to examine the baseline behavioral parameters with all animals. In the next 3 weeks, mice were exposed to the CVS. Our chronic stress paradigm was a modified version of one described previously (Sterrenburg et al., 2011) and consisted of six stressors (shaking the animals within their home cage on a shaker for 2 h; overnight illumination for 12 h; cage tilting (45°) for 12 h; housing on wet bedding for 12 h; restraint in 50 ml plastic tubes with holes for 2 h; exposure to cold (4 °C) by placing mice into a refrigerator for 30 min), two of them were used daily. Mice in the control groups were handled in the same way as