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Somatostatin receptor subtype 4 activation is involved in anxiety and depression-like behavior in mouse models

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ABSTRACT

Somatostatin regulates stress-related behavior and its expression is altered in mood disorders. However, little is known about the underlying mechanisms, especially about the importance of its receptors (sst_1–sst_5) in anxiety and depression-like behavior. Here we analyzed the potential role of sst_4 receptor in these processes, since sst_4 is present in stress-related brain regions, but there are no data about its functional relevance. Genetic deletion of sst_4 (*Sstr4*$^{-/-}$) and its pharmacological activation with the newly developed selective non-peptide agonist J-2156 were used. Anxiety was examined in the elevated plus maze (EPM) and depression-like behavior in the forced swim (FST) and tail suspension tests (TST). Neuronal activation during the TST was monitored by Fos immunohistochemistry, receptor expression was identified by sst_4^{LacZ} immunostaining in several brain regions. *Sstr4*$^{-/-}$ mice showed increased anxiety in the EPM and enhanced depression-like behavior in the FST. J-2156 (100 μg/kg i.p.) exhibited anxiolytic effect in the EPM and decreased immobility in the TST. J-2156 alone did not influence Fos immunoreactivity in intact mice, but significantly increased the stress-induced Fos response in the dorsal raphe nucleus, central projecting Edinger–Westphal nucleus, periaqueductal gray matter, the magnocellular, but not the parvocellular part of the hypothalamic paraventricular nucleus, lateral septum, bed nucleus of the stria terminalis and the amygdala. Notably, sst_4^{LacZ} immunoreactivity occurred in the

Abbreviations: EPM, elevated plus maze test; OFT, open field test; FST, forced swim test; TST, tail suspension test; i.p., intraperitoneal; dRN, the dorsal raphe nucleus; EWcp, the central projecting Edinger–Westphal nucleus; dPAG and lPAG, respectively, the dorsal and lateral parts of the mesencephalic periaqueductal gray matter; pPVN and mPVN, respectively, the parvocellular and magnocellular parts of the paraventricular nucleus of the hypothalamus; vLS and dLS, respectively, the ventral and dorsal lateral septum; ovBST, dlBST, dmBST and vBST, respectively, the oval, dorsolateral, dorsomedial and ventral nuclei of the bed nucleus of the stria terminalis; CeA, BLA and MeA, respectively, the central, basolateral and medial amygdaloid nuclei.

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central and basolateral amygdala. Together, these studies reveal that sst_4 mediates anxiolytic and antidepressant-like effects by enhancing the stress-responsiveness of several brain regions with special emphasis on the amygdala.

1. Introduction

Stress-related mental disorders, mainly depression and anxiety are among the most frequent medical burdens in Western societies, leading to a strong deterioration of the ability to function and the increase of mortality (Ebmeier et al., 2006). Since depression is one of the leading causes of disability, the research of its pathophysiological mechanisms and potential drug targets is essential. Although several aspects have been revealed, the exact underlying mechanisms are still not fully understood.

The wide expression of somatostatin and its receptors in brain areas regulating the stress response, such as the amygdala (Viollet et al., 2008) suggests that this neuromodulator peptide is involved in emotional regulation. This was proven by several recent clinical and experimental results emphasizing the importance of somatostatin in stress-related behavior and the pathogenesis of mood disorders (for review see Martel et al., 2012). In clinical studies, decreased somatostatin levels have been found in the dorsolateral prefrontal cortex (Sibille et al., 2011), the cingulate cortex (Tripp et al., 2011) and the amygdala (Guilloux et al., 2012) of patients with major depression. Somatostatin levels were also shown to be reduced in chronically stressed rats (Czéh et al., 2015). It has recently been shown that the switch between dopamine and somatostatin expression in hypothalamic interneurons regulates the behavioral consequences of altered light–dark cycle (Dulcis et al., 2013). These data suggest that the reduction of inhibitory neurotransmission, including the somatostatinergic systems, is strongly involved in the pathophysiology of mood disorders and anxiety (Lin and Sibille, 2013). Somatostatin release increases in the amygdala (Brodin et al., 1994), hypothalamus (Arancibia et al., 2000) and hippocampus (Arancibia et al., 2001) after acute stress. Somatostatin gene-deficient mice show increased behavioral emotionality, elevated basal corticosterone and gene expression changes similar to those seen in depressed patients together with altered interneuronal transcriptome profile (Lin and Sibille, 2015). Intracerebroventricularly administered somatostatin in rats exerted anxiolytic and antidepressant-like effects in the elevated plus maze and forced swim tests, and suppressed the hippocampal theta activity (Engin et al., 2008). In the anxiolytic activity of the peptide, the amygdala and the septum are the most important brain areas, since its selective injection into these structures exerts the same amount of effect than the intracerebroventricular infusion (Yeung et al., 2011). There are also data suggesting the role of somatostatin in the antidepressant action of the tricyclic antidepressant imipramine (Nilsson et al., 2012).

In spite of the well-established effect of somatostatin on the activity of neurons via five subtypes of G protein-coupled somatostatin receptors (sst_1–sst_5) (Hoyer et al., 1995), little attention has been given to the relevance of these receptors in behavioral effects of the peptide. The role of sst_2 and sst_3 receptors have been shown in the anxiolytic and antidepressant-like effects of somatostatin, since the treatment with synthetic sst_2 and sst_3 agonists produced similar effects to the native peptide (Engin and Treit, 2009). Additionally, the anxiolytic action of somatostatin could be reversed with the intra-amygdalar and intra-septal microinfusions of an sst_2 antagonist (Yeung and Treit, 2012). The *Sstr2* gene-deleted mice showed increased anxiety and pituitary ACTH release (Viollet et al., 2000). Acute psychological stress induced a selective increase of sst_2 mRNA expression in the amygdala (Nanda et al., 2008). In contrast, the relevance of other somatostatin receptors in physiological and pathological emotional regulation is not known.

According to immunohistochemical studies, the sst_4 receptor is widely distributed in several brain regions involved in the regulation of mood, anxiety and stress, such as the cortex, striatum, hippocampus and the amygdala (Schreff et al., 2000; Selmer et al., 2000a, 2000b), but yet very little is known about its functional relevance. The only evidence for its importance in the brain was obtained with the sst_4 agonist NNC 26-9100. It exerted protective actions against cognitive decline in a transgenic mouse model of Alzheimer's disease (Sandoval et al., 2011).

In the present study we applied *Sstr4* knockout mice ($Sstr4^{-/-}$), highly selective sst_4 agonist, J-2156, behavioral tests and quantitative immunohistochemistry to examine the role of sst_4 in anxiety and depression-like behavior shown in acute stressful situations. J-2156 is a small molecular peptidomimetic drug binding with nanomolar affinity to sst_4 and showing 400-fold higher selectivity to sst_4 than to the other receptors. In a G-protein activation assay J-2156 showed 2.5 times greater activity as native somatostatin (Engström et al., 2005). Based on these properties, the authors identified J-2156 as a "superagonist". To gain some information about the mechanisms of the effect exerted by J-2156 on stress-related behavior, we examined the changes of Fos immunoreactivity in response to TST and J-2156 administration in several stress-related cerebral structures. Fos is a widely used neuronal activation marker, the product of an immediate early gene, *cfos* (Kovács, 1998). Finally, to show the presence of sst_4 in the nuclei of amygdala, sst_4^{LacZ} immunohistochemistry in $Sstr4^{-/-}$ animals was performed.

2. Materials and methods

2.1. Animals

We used *Sstr4* gene-deleted ($Sstr4^{-/-}$) and wildtype ($Sstr4^{+/+}$) mice generated on C57Bl/6J background, and also C57Bl/6J and CD1 mice for investigating the effects of the agonist. We worked exclusively with male animals (5–6 months old) bred in the Laboratory Animal House of the Department of Pharmacology and Pharmacotherapy of the University of Pécs, kept in standard plastic cages at 24–25 °C, under a 12–12 h light–dark cycle and provided with standard rodent chow and water *ad libitum*. Behavioral tests and perfusion were performed in the morning.

The original breeding pairs of heterozygous mice ($Sstr4^{+/-}$) were generated and kindly donated by the group of Dr. Pierce C. Empson (Laboratory of Molecular Neuroscience, The Babraham Institute, Babraham Research Campus, Babraham, Cambridge CB22 3AT, United Kingdom). *Sstr4* deletion was performed by the replacement with a construct containing the *LacZ* gene (for more details see Helyes et al., 2009). $Sstr4^{+/-}$ animals were paired and the genotype of their offsprings was determined with PCR. $Sstr4^{-/-}$ and $Sstr4^{+/+}$ mice were then bred separately.

C57Bl/6J and CD1 mice were purchased from the Charles River Hungary Ltd.

All experimental procedures complied with the recommendations of the 1998/XXVIII Act of the Hungarian Parliament on Animal