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Chronic venlafaxine treatment fails to alter the levels of galanin system transcripts in normal rats

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ABSTRACT

It is widely accepted that efficacy and speed of current antidepressants' therapeutic effect are far from optimal. Thus, there is a need for the development of antidepressants with new mechanisms of action. The neuropeptide galanin and its receptors (GalR1, GalR2 and GalR3) are among the promising targets. However, it is not clear whether or not the galanin system is involved in the antidepressant effect exerted by the currently much used inhibitors of the reuptake of serotonin and/or noradrenaline. To answer this question we administered the selective serotonin and noradrenaline reuptake inhibitor (SNRI) venlafaxine (40 mg/kg/day via osmotic minipumps) to normal rats and examined the levels of the transcripts for galanin and GalR1–3 after a 3-week venlafaxine treatment in the dorsal raphe, hippocampus and frontal cortex. These areas are known to be involved in the effects of antidepressants and in depression itself. Venlafaxine failed to alter the expression of any of the galanin system genes in these areas. Our results show that one of the most efficient, currently used SNRIs does not alter transcript levels of galanin or its three receptors in normal rats. These findings suggest that the pro- and antidepressive-like effects of galanin reported in animal experiments may employ a novel mechanism(s).

1. Introduction

Major depressive disorder (MDD) is amongst the most debilitating diseases of modern societies (Han and Wang, 2005; Kessler et al., 2006). While pharmacological treatments do exist, they lack effectiveness in many individuals (O'Leary et al., 2015; Pigott et al., 2010; Thase et al., 2005; Trivedi et al., 2006), they cannot always prevent further depressive episodes (Pigott et al., 2010; Trivedi et al., 2006) and they have a delay in the onset of their therapeutic effect (Gex-Fabry et al., 2004; Machado-Vieira et al., 2008; O'Leary et al., 2015; Smith et al., 2002). Thus, research is ongoing for discovering better antidepressant treatments and new possible targets (O'Leary et al., 2015). In these efforts the galanin system has emerged as a candidate for further exploration (Counts et al., 2008; Elvander et al., 2004; Holets et al., 1988; Lang et al., 2015; Pieribone et al., 1998; Senut et al., 1989; Skofitsch et al., 1986; Weiss et al., 1998; Xu and Hokfelt, 1997).

Galanin is a 29 amino acid long neuropeptide (Tatemoto et al., 1983) acting via three G-protein coupled receptors, Gal1, Gal2 and Gal3 (Barreda-Gomez et al., 2014; Branchek et al., 2000; Lang et al., 2015). Galanin and the Gal1–3 receptors are widely distributed in the rat (Melander et al., 1986; O'Donnell et al., 2002; Skofitsch and Jacobowitz, 1985; Skofitsch et al., 1986) and human (Kohler et al., 1989; Kordower et al., 1992) brain, and among their many functions is the modulation of the brain's serotonin (5-HT) and noradrenaline (NA) neurons (Fuxe et al., 1998; Pieribone et al., 1998; Weiss et al., 1998). This was further supported by the fact that all three receptors are found in the dorsal raphe (DR) and locus coeruleus (LC) of rats (Burazin et al., 2000; Mennicken et al., 2002; O'Donnell et al., 1999, 2002) and that galanin is co-expressed in almost 40% of the serotonergic neurons in the DR (Xu and Hokfelt, 1997) and in around 80% of the noradrenergic neurons in the LC (Holets et al., 1988). However, reports indicate that galanin also modulates GABAergic (Sharkey et al., 2008) and cholinergic neurotransmission (Counts et al., 2008; Elvander et al., 2004; Senut et al., 1989) and may thus directly and indirectly influence functions in distinct regions, such as the hippocampus (HC) and frontal cortex (FC). Functionally, galanin has been suggested to be implicated in numerous pathological states and physiological processes in the central

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nervous system, like learning and memory (Brewer et al., 2005), epilepsy (Kovac and Walker, 2013; Lerner et al., 2008), addiction (Picciotto, 2010) or pain perception (Xu et al., 2008), but particular focus has been on depressive states and anxiety-related disorders/behaviors (Fuxe et al., 1998; Kuteeva et al., 2008a, 2008b; Lu et al., 2007; Murck et al., 2004). Indeed, studies showed that a Gal3 antagonist (Swanson et al., 2005) and a Gal2 agonist (Kuteeva et al., 2008b; Lu et al., 2005; Saar et al., 2013a, 2013b) attenuated depressive symptoms in rodent models of depression, while recent association studies by Wray et al. (2012) and Juhasz et al. (2014) have underlined the importance of polymorphisms in the galanin genes in humans. However, increase in galanin release may exert opposite effects on depression related phenotypes. In general terms, reports cited above indicate that activation of Gal1 and Gal3 causes depression-like effects, while blockade of these receptors and activation of Gal2 were associated with antidepressant-like effects in the behavior of rats.

The galanin-like peptide precursor (Galp) encodes two transcripts related to the galanin system which are formed through splicing. One, Galp, was isolated from porcine hypothalamus and considered originally as a possible Gal2 agonist (Ohtaki et al., 1999). Later its agonistic properties have also been demonstrated for Gal1 and Gal3 in transfected cells, and results from knock-out mice raised the possibility of a so far undiscovered native receptor for the peptide (Krasnow et al., 2004; Lang et al., 2005, 2015). The expression of Galp is restricted to specific cells in the hypothalamus, its release is regulated by e.g. insulin and leptin (Jureus et al., 2000, 2001) and once released, it influences feeding behavior and thermogenesis (Hansen et al., 2003; Lawrence et al., 2002; Matsumoto et al., 2002). While feeding irregularities may accompany mood disorders, so far, no link was demonstrated between Galp and depression/antidepressant actions.

The other transcript is alarin, a result of alternate splicing of Galp, which contains 25 amino acids (Santic et al., 2006, 2007). Alarin cannot bind to Gal1–3 and has no identified receptors so far (Lang et al., 2015), but is considered as a galanin system peptide because of its origin. Its distribution in the rodent brain contrasts that of the galanin-like peptide: it is abundant in cortical layers, the HC and the LC in mice among many other areas (Eberhard et al., 2012). Functionally alarin is involved in the regulation of feeding behavior and the modulation of the hypothalamus–pituitary–adrenal (HPA) axis (Fraley et al., 2012; Van Der Kolk et al., 2010). In addition, alarin has been implicated in depression by the recent studies of Wang et al., who proposed central roles for the modulation of hypothalamic hormones, the brain-derived neurotrophic factor and TrkB in its anti-depressive effects (Wang et al., 2014, 2015).

In rats, chronic fluoxetine (FLX) and paroxetine (PRX) treatments, which belong to the selective serotonin reuptake inhibitor (SSRI) class of antidepressants, elevated galanin expression in the DR (Lu et al., 2005; Rovin et al., 2012). In mice, chronic sertraline, another SSRI, or FLX treatments were able to induce similar changes in the HC region (Christiansen et al., 2011; Yamada et al., 2013). At the same time, chronic treatment with phenelzine (PLZ), a monoamino-oxidase inhibitor (MAOI) failed to cause any alterations in the gene expression of galanin and its receptors in the DR of rats (Rovin et al., 2012). The latter result suggests that the effects on galanin and Gal1–3 are not uniform among antidepressants and may be related to certain pharmacological properties. However, neither galanin, nor galanin receptor or Galp gene expression was studied after administration of a serotonin-noradrenaline or selective noradrenaline reuptake inhibitor antidepressant.

Venlafaxine (VLX) belongs to a group of drugs with an extended mechanism of action compared to SSRIs, that is they selectively inhibit both serotonin and noradrenaline reuptake (SNRIs). It has been used widely in clinical practice and been proven to be more effective than SSRIs in terms of economic costs, remission rates and earlier onset of antidepressant actions (Gex-Fabry et al., 2004; Smith et al., 2002). VLX has been shown to activate expression of a number of genes, for example such involved in neurotrophic signaling, glutamatergic transmission, neuroplasticity, synaptogenesis and cognitive processes (Tamasi et al., 2014). Interactions between noradrenergic neurotransmission and galanin signaling have been already discussed in the literature [for reviews see (Lu et al., 2007)], while those with the serotonergic system are described above. Thus, it is tempting to speculate that, if SSRIs are able to modulate galanin signaling, this may also be the case with SNRIs, in fact the effect could be enhanced.

In this report we present the results of a gene expression analysis of the galanin, Gal1, Gal2 and Gal3 and Galp genes from the DR, HC and FC regions of Dark Agouti (DaAg) rats following 3-week long chronic VLX treatment.

2. Materials and methods

The presented experiment was part of a large-scale microarray study, thus the methods used in this study are discussed in details in (Petschner et al., 2013; Tamasi et al., 2014), and here we only provide a brief description.

2.1. Animals and drugs

In this study altogether 20 male, DaAg rats (Harlan, Olac Ltd., Shaw's Farm, Blackthorn, Bicester, Oxon, UK, aged: 8 weeks, weighing 126.85 ± 4.22 g (mean ± S.E.M.) were used. The animal experiments and housing conditions were carried out in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC), the National Institutes of Health Principles of Laboratory Animal Care (NIH Publication 85-23, revised 1985) and special national laws (the Hungarian Governmental Regulation on animal studies, 31 December 1998 Act). The experiments were approved by the National Scientific Ethical Committee on Animal Experimentation and permitted by the Food Chain Safety and Animal Health Directorate of the Central Agricultural Office, Hungary (permission number: 22.1/3152/001/2007).

VLX was dissolved in 0.9% NaCl solution, and Alzet 2001 osmotic minipumps (Durect Corp., CA, USA) were filled with the solution.

2.2. Drug administration and experimental design

Alzet osmotic minipumps were inserted subcutaneously under the back skin of the rats in the VLX-groups while the control group underwent sham surgery. All surgery was performed under anesthesia with halothane. Due to the limited volume of the osmotic pumps, the sham surgery and minipump insertion had to be repeated every week for 3 weeks. The pumps delivered 40 mg/kg VLX each day, while food and water were available ad libitum. During surgical procedures one animal died, thus, altogether 19 animals were used in the experiments.

2.3. RNA extraction and sample preparation

Three weeks after the first osmotic minipump insertion rats were killed quickly by decapitation. The brains were removed, approximately 2 mm thick coronal sections were cut and the HC, FC and DR regions were dissected according to the Atlas of Paxinos and Watson (Paxinos and Watson, 1986) as follows: dorsal HC: from bregma −2.5 mm to −4.5 mm; FC: from bregma +1.7 to −0.3 mm; DR: from bregma −7 mm to −8 mm, respectively, and stored at −80 °C. The samples were homogenized with 1 ml TRIzol reagent and RNA was isolated. The pellets were dissolved in 20 μl diethylpyrocarbonate treated-dH2O (DEPC-dH2O) and solutions stored at −80 °C. The optical density (OD) ratios were determined for all samples for quality check and randomly repeated to evaluate the reliability of the measurements (no significant difference was observed, data not shown). Thereafter two-two randomly selected samples from the same treatment groups and region with the best OD ratios were pooled, resulting in altogether four pooled samples per treatment and region. From VLX treated and vehicle treated pools microarray experiments were performed by