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Research report

Dorsal hippocampal galanin modulates anxiety-like behaviours in rats

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ABSTRACT

Galanin, a peptide expressed in mammalian brain regions, has been implicated in anxiety and depression. Galanin signalling occurs through three G protein-linked receptors (GAL_1, GAL_2 and GAL_3). Galanin regulates the release of neurotransmitters in some brain regions related to anxiety, including the hippocampus. GAL_2 is the most abundant galanin receptor in the dorsal hippocampus. In this study, we evaluated whether galanin administered in the dorsal hippocampus affected anxiety-like behaviours of rats. We also investigated if GAL_2 receptors are involved in the anxiogenic-like effect of galanin using a GAL_2 antagonist, M871. To achieve these objectives, male adult Wistar rats received intra-dorsal hippocampal delivery of galanin (0.3 and 1.0 nmol/0.5 μl) or vehicle in experiment 1 and GAL_2 antagonist M871 (1.0 and 3.0 nmol/0.5 μl) or vehicle in experiment 2. Twenty min after administration of drugs, the animals were tested in the elevated plus-maze (EPM). Galanin (1.0 nmol) induced anxiogenic-like behaviours, while the GAL_2 receptor antagonist M871 (3.0 nmol) induced anxiolytic-like behaviours in rats exposed to the EPM, indicating a tonic effect of galanin. In experiment 3, we evaluated whether previous infusion of the GAL_2 antagonist M871 (1 or 2 nmol) in the dorsal hippocampus would block the anxiogenic-like effect of galanin in rats tested in the EPM. We showed that M871 (2.0 nmol) counteracted the anxiogenic-like effect of galanin infused in the dorsal hippocampus of rats. Altogether, our results provide evidence that galanin promotes pharmacological and tonic anxiogenic-like effects in the dorsal hippocampus, possibly mediated by GAL_2 receptors.

1. Introduction

According to the National Institutes of Health (National Institute of Mental Health), occasional anxiety caused by worry or fear is a normal emotion. However, in patients with an anxiety disorder, anxiety does not disappear and may worsen over time or interfere with daily activities, such as work, school, and relationships. The classification of anxiety disorders includes separation anxiety disorder, selective mutism, specific phobia, social anxiety disorder, panic disorder, agoraphobia and generalized anxiety disorder (GAD) (American Psychiatric Association, 2013). Despite some limitations, many animal models are used to attempt assessing anxiety in order to understand the neurobiological mechanisms underlying anxiety disorders as well as to discover new targets for the treatment of these heterogeneous conditions (Kalueff et al., 2007; Bouwknecht, 2015).

An abundance of evidence suggests that galanin, a neuropeptide widely expressed in the mammalian brain (Melander et al., 1986; Barreda-Gómez et al., 2014; Lang et al., 2015), modulates anxiety-like (Karlsson and Holmes, 2006; Zhao et al., 2013) and depression-like (Kuteeva et al., 2010; Millón et al., 2017) behaviours. Three galanin receptor subtypes mediate the actions of galanin, and they are all G-protein coupled receptors (GPCR). GAL_1 and GAL_3 are usually coupled to the $G_{i/0}$ protein and induce membrane hyperpolarization (Branchek et al., 2000; Smith et al., 1998; Wang et al., 1998). GAL_2 is often coupled to $G_{q/11}$ and increases intracellular Ca^{++} (Branchek et al., 2000; Fathi et al., 1998; Wang et al., 1998), though it may also be coupled to $G_{i/0}$ (Lang et al., 2015). Galanin receptors regulate the release of several neurotransmitters. For example, galanin decreased glutamate release in the arcuate nucleus of the hypothalamus (Kinney et al., 1998) and in ventral hippocampal slices (Zini et al., 1993). Additionally, galanin raised extracellular levels of noradrenaline in the medial prefrontal cortex (Yoshitake et al., 2013).

The effects of galanin on anxiety may depend on the site of drug administration, on the galanin receptor subtypes activated and on the animal models of anxiety employed (Barrera et al., 2005; Holmes and Picciotto, 2006; Soares et al., 2016). For instance, administration of galanin in the central amygdala induced an anxiogenic-like effect in rats evaluated in a modified Vogel test but not in the elevated plus-maze (EPM) (Moller et al., 1999). Infusion of galanin in the dorsal periaqueductal grey (Soares

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et al., 2016) or in the dorsal raphe nucleus (DRN) (Silote et al., 2013) impaired inhibitory avoidance of rats in the elevated T-maze, suggesting an anxiolytic-like effect. Additionally, intra-DRN administration of a selective GAL_1 agonist (M617) enhanced anxiety-like behaviour in rats tested in the elevated T-maze, while administration of a preferential GAL_2 agonist (AR-M1896) reduced this behaviour (Morais et al., 2016).

Although some studies have implicated galanin in the modulation of learning, memory, neuroprotection and epilepsy (Elliott-Hunt et al., 2004; Mazarati et al., 2006; Mazarati and Lu, 2005; Ögren et al., 1998; Schott et al., 2000) in the hippocampus, to our knowledge, no previous study examined if this neuropeptide also affects anxiety-like behaviours in the dorsal hippocampus. The well-known involvement of dorsal hippocampus in memory and learning processes outshined its participation in anxiety (Graeff, 1997). Nevertheless, results from anatomical, behavioural and pharmacological studies support the role of dorsal hippocampus in modulating anxiety (Andrade et al., 2013; File et al., 2000). For instance, the dorsal hippocampus receives noradrenergic and serotonergic inputs from locus coeruleus (Page and Abercrombie, 1999) and from median raphe nucleus (Azmitia, 1981), respectively. Exposure to aversive stimuli increased noradrenaline and serotonin (Kalén et al., 1989) while exposure to novelty increased acetylcholine in the hippocampal formation (Giovannini et al., 2001). The infusion of 8-OH-DPAT, a $5\text{-}HT_{1A}$ receptor agonist, in the dorsal hippocampus facilitated inhibitory avoidance acquisition in rats tested in the elevated T-maze (Dos Santos et al., 2008). Also, temporary pharmacological inhibition of neurons from dorsal hippocampus enhanced anxiety-like behaviours in rats tested in the elevated plus-maze (Zhang et al., 2014).

The presence of galanin and its receptors, as well as its ability to regulate the release of neurotransmitters in the hippocampus, suggests that galanin signalling may also modulate behaviours related to anxiety in this brain region. Galanin immunoreactivity and binding sites have been demonstrated in the hippocampal formation, especially in the dorsal dental gyrus (Skofitsch and Jacobowitz, 1986; Xu et al., 2005). The hippocampus expresses at least GAL_1 and GAL_2 receptors, although GAL_2 is the most abundant galanin receptor in the dorsal hippocampus (O'Donnell et al., 1999; Lu et al., 2005). Most galanin-positive fibres in the dorsal hippocampus overlap with noradrenergic terminals originating in the locus coeruleus (Xu et al., 1998). Additionally, galanin raises acetylcholine levels in the dorsal hippocampus, likely through GAL_2 receptor activation (Ögren et al., 1998; Yoshitake et al., 2011).

Thus, we investigated whether infusion of galanin in the dorsal hippocampus mediates anxiety-like behaviours in rats. For this purpose, we chose a widely used behavioural test, the EPM, to evaluate anxiety-related drug effects (Walf and Frye, 2007). Additionally, using a pharmacological approach, we examined the involvement of GAL_2 receptors in the anxiogenic-like effect of galanin in the dorsal hippocampus. Although the selectivity of peptidergic galanin receptor ligands is a matter of concern (Kuteeva et al., 2008), *in vitro* and *in vivo* studies support the view that M871 acts as a GAL_2 antagonist (Liu et al., 2001; Lundström et al., 2005; Sollenberg et al., 2006). First, we evaluated if M871 administered in the dorsal hippocampus would affect the anxiety-like behaviour of rats in the EPM. Finally, we tested whether pretreatment with the GAL_2 antagonist M871 would block the anxiogenic-like effect of galanin in the dorsal hippocampus.

2. Results

2.1. Dorsal hippocampus microinjection sites

Injection sites in the dorsal hippocampus of each experiment are shown in a diagrammatic representation in Fig. 1. Rats with

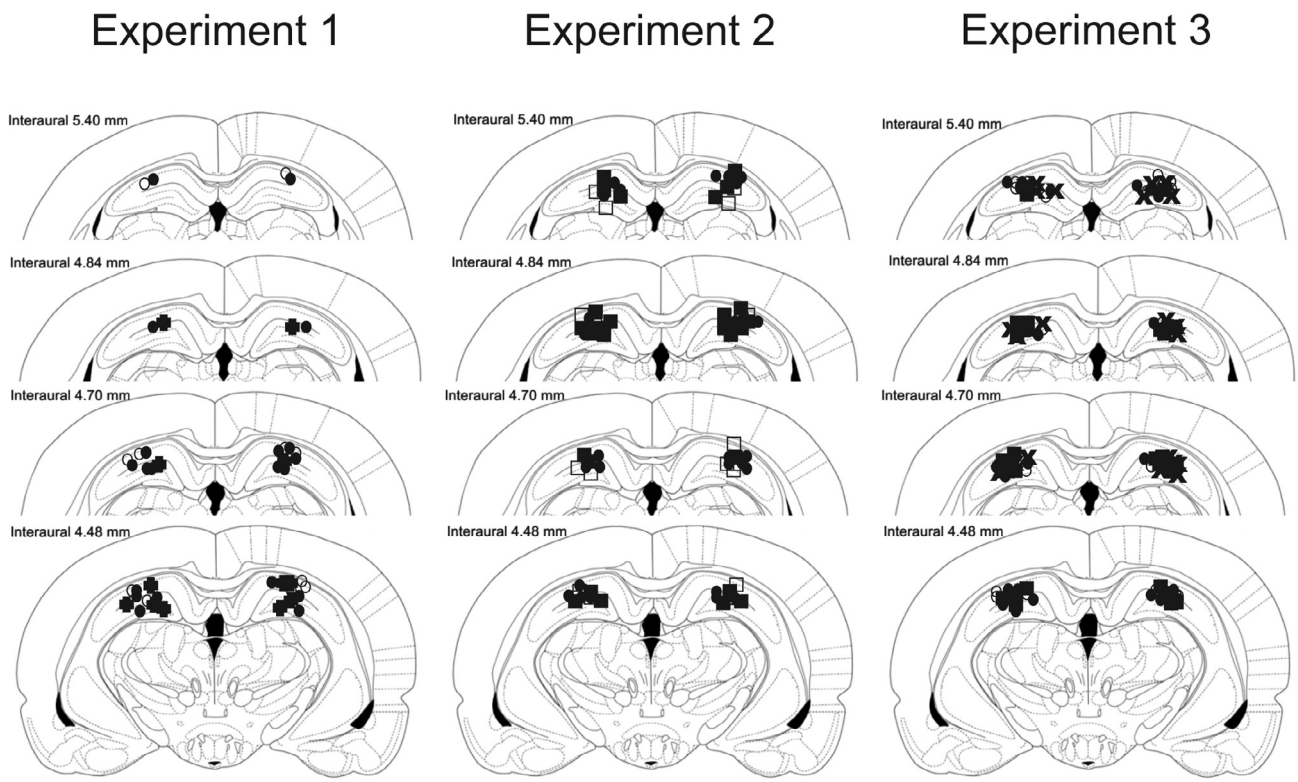

Fig. 1. A diagrammatic representation based on the rat brain atlas of Paxinos and Watson (2008) indicating injections sites of saline (●), galanin 0.3 nmol (◖), galanin 1 nmol (○), M871 1 nmol (■), M871 3 nmol (□), M871 1 nmol/Gal 1 nmol (✖) from experiments 1–3.