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Research Paper

Expression of galanin and its receptors are perturbed in a rodent model of mild, blast-induced traumatic brain injury

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ABSTRACT

The symptomatology, mood and cognitive disturbances seen in post-traumatic stress disorder (PTSD) and mild blast-induced traumatic brain injury (mbTBI) overlap considerably. However the pathological mechanisms underlying the two conditions are currently unknown. The neuropeptide galanin has been suggested to play a role in the development of stress and mood disorders. Here we applied bio- and histochemical methods with the aim to elucidate the nature of any changes in the expression of galanin and its receptors in a rodent model of mbTBI. In situ hybridization and quantitative polymerase chain reaction studies revealed significant, injury-induced changes, in some cases lasting at least for one week, in the mRNA levels of galanin and/or its three receptors, galanin receptor 1–3 (GalR1–3). Such changes were seen in several forebrain regions, and the locus coeruleus. In the ventral periaqueductal gray GalR1 mRNA levels were increased, while GalR2 were decreased. Analysis of galanin peptide levels using radioimmunoassay demonstrated an increase in several brain regions including the locus coeruleus, dorsal hippocampal formation and amygdala. These findings suggest a role for the galanin system in the endogenous response to mbTBI, and that pharmacological studies of the effects of activation or inhibition of different galanin receptors in combination with functional assays of behavioral recovery may reveal promising targets for new therapeutic strategies in mbTBI.

1. Introduction

The high incidence of traumatic brain injury (TBI), including blast-induced TBI (bTBI), is a significant civilian and military health issue (Chapman and Diaz-Arrastia, 2014; Hoge et al., 2004; Kennedy et al., 2010). In the context of blast exposure, the rapid changes associated with the pressure wave alone are believed to result in blast-induced TBI (bTBI), as the initial burst of energy is transmitted through the head, into the brain (Risling and Davidsson, 2012). In the current conflict zones 75–85% of injuries are associated with explosive weaponry, of these 80–95% are mild (mbTBI) (Hoge et al., 2008; MacGregor et al., 2011; Warden, 2006).

Clinically, the neurological Glasgow Coma Scale is used to assess the severity of TBI (Mckee and Daneshvar, 2015). Furthermore, post-traumatic amnesia following the incident should last no longer than 24 h, and loss of consciousness should not exceed 30 min (Ruff et al., 2009). There are minimal, if any structural changes to the brain, with negative findings on the most commonly utilized imaging modalities, such as computed tomography (CT) scans, and magnetic resonance imaging (MRI) (Drake et al., 2010).

Despite the limited gross pathology of mbTBI, a range of cognitive and emotional problems may develop post-injury, affecting attention and decision making and leading to memory deficits, increased irritability, aggression, anxiety, and depression (Brenner et al., 2009; Hoge et al., 2004; Kennedy et al., 2010; Kok et al., 2012). The majority of these symptoms resolve themselves, while for around a third symptoms may persist for months or even years, and thus are life debilitating (Kennedy et al., 2010; Okie, 2005).

Much of the symptoms of mbTBI are also leading indicators of post-traumatic stress disorder (PTSD) caused by exposure to extreme

Abbreviations: AADC, aromatic acid decarboxylase; Amg, amygdala; CCK, cholecystokinin; CX, occipital cortex; d/vHiFo, dorsal/ventral hippocampal formation; DRN, dorsal raphe nucleus; ERC, entorhinal cortex; Gal R1-3, galanin receptor subtype 1–3; Hyp, hypothalamus; ISH, in situ hybridization; LC, locus coeruleus; MGD, mean gray density; NPY, neuropeptide tyrosine; PFC, prefrontal cortex; PTSD, post-traumatic stress disorder; qPCR, quantitative polymerase chain reaction; RIA, radioimmunoassay; TBI, traumatic brain injury; bTBI, blast-induced TBI; mbTBI, mild bTBI; TH, tyrosine hydroxylase; TPH, tryptophan hydroxylase; vPAG, ventral periaqueductal gray; MRI, magnetic resonance imaging; CT, computed tomography; D, day; NA, noradrenaline; 5-HT, 5-hydroxytryptamine; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

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psychological stress, frequently without physical injury. However, PTSD may also involve a re-experiencing phenomena of the initial event and avoidance or hypervigilance in response to even very distantly related stimuli (Sherin and Nemeroff, 2011). These symptoms may occur immediately or after months to years post-trauma. Hence in the absence of objective criteria, the differential diagnosis between mbTBI and PTSD is challenging (Davenport et al., 2015; Schneiderman et al., 2008). PTSD and mbTBI may, in fact be two ends of a spectrum disorder with partly overlapping molecular pathologies (Kwon et al., 2011).

We have previously reported on the effect of a single blast on behavior and on some of the monoaminergic systems (Kawa et al., 2014). We found an increase in climbing on day (D)1 in the forced swim test as well as a transient, short lasting elevation in noradrenaline (NA) levels in a number of forebrain regions, and in transcripts for the two rate-limiting enzymes tyrosine hydroxylase (TH) and tryptophan hydroxylase 2 (TPH2) in the lower brain stem.

The focus in the present study is on the 29 amino acid (30 in human) neuropeptide galanin (Tatemoto et al., 1983), which in rat is co-expressed in the NA neurons in the locus coeruleus (LC) and in the serotonin (5-hydroxytryptamine, 5-HT) neurons in the dorsal raphe nucleus (DRN) (Fuxe et al., 1990; Holets et al., 1988; Melander et al., 1986b; Xu and Hökfelt, 1997; Xu et al., 1998a, 1998b). Functionally, galanin is known to, for example, modulate 5-HT signaling (Fuxe et al., 1998), as well as inhibit 5-HT and NA release (Yoshitake et al., 2003) and neuronal firing of NA neurons in the LC (Pieribone et al., 1998; Seutin et al., 1989; Sevcik et al., 1993; Xu et al., 2005). Galanin mediates its actions through three G-protein coupled receptor subtypes, galanin receptor 1–3 (GalR1–3) (Branchek et al., 2000; Habert-Ortoli et al., 1994; Lang et al., 2015). These receptors are widely expressed in the rat brain (O'Donnell et al., 2003; Waters and Krause, 2000). There is evidence for a role of galanin in anxiety- and mood-related disorders (Barrera et al., 2005; Fuxe et al., 1991, 1998; Holmes and Picciotto, 2006; Karlsson and Holmes, 2006; Kuteeva et al., 2010; Lu et al., 2007; Picciotto et al., 2010; Sciolino and Holmes, 2012; Weiss et al., 1998, 2005).

Here we assessed the effect of the blast on the galanin system using in situ hybridization (ISH), quantitative polymerase chain reaction (qPCR), and radioimmunoassay (RIA). The brain regions analyzed were selected based on the extensive literature, implicating them in the pathobiology of mbTBI and overlapping disorders such as PTSD (Golub et al., 2011; Kwon et al., 2011; Sherin and Nemeroff, 2011; Simmons and Matthews, 2012; Tovote et al., 2015). Since our previous ISH results were obtained from the same rats used in the present study, direct comparisons between the monoamine and galanin systems are possible.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (Taconic, Ry, Denmark), 10–12 weeks old and weighing 290–340 g, were used. All experiments were performed in accordance with the Swedish National Guidelines for Animal Experiments, and approved by the Stockholm Animal Care and Use Ethics Committee (Stockholm Norra Djurförsöksetiska Nämnd).

2.2. Experimental groups and manipulations

This study is composed of three separate experiments, each with different rats assigned to two groups, sham or exposed. All rats were anesthetized by isoflurane, Dormicum® and Hypnorm® (Fentanyl/fluaniscane, Janssen, Stockholm, Sweden).

Experiment #1 was ISH, where a total of thirty-three rats were terminated at four post-exposure time-points: 2 h (n = 5), day (D) 1 (n = 5), D3 (n = 4) and D7 (n = 8), and a total of 11 shams were used. Experiment #2 consisted of qPCR analysis, and a total of twenty rats were sacrificed at D1 (6 exposed and 4 sham) and D7 (5 exposed and 5 sham). Experiment #3 was a RIA analysis with a total of twenty-one rats sacrificed at D3 (5 exposed and 5 sham) and D7 (6 exposed and 5 sham).

2.3. Exposure model

Rats were exposed to a single explosion using a blast tube, causing a mild TBI as described (Kawa et al., 2014; Risling et al., 2011). It should be noted that this is a model for isolated primary blast TBI caused by the overpressure from the detonation, and does not involve rotational acceleration, which is the usual mechanism for mild TBI in contact sports or fall accidents.

2.4. In situ hybridization (ISH)

The rats were anesthetized and decapitated, the brains removed, placed on dry ice and stored at −70 °C until use. Serial coronal, 14 μm thick sections were cut using Cryo-Star HM 560 M (MICROM International GmbH, Heidelberg, Germany).

Oligonucleotides complementary to rat mRNA for galanin, neuropeptide tyrosine (NPY) and aromatic acid decarboxylase (AADC) (Table A.1) were labeled with deoxyadenosine 5′triphosphate α–P^{32} (Perkin Elmer, Boston, MA) at the 3′-end using terminal deoxynucleotidyltransferase.

RNA probes specific to GalR1 were prepared from rat hypothalamus cDNA (Table A.1). The PCR fragments were then subcloned into PCR1II-TOPO vector (Life Technologies, Carlsbad, CA) and transcribed using T7 and SP6 RNA polymerases to generate sense and antisense RNA probes. Hybridization, post-hybridization conditions, and further details are described in the Appendices.

2.5. Quantitative polymerase chain reaction (qPCR)

The following regions were dissected using anatomical landmarks (Paxinos and Watson, 2007): prefrontal cortex (PFC), occipital cortex (CX), entorhinal cortex (ERC), hypothalamus (Hyp), amygdala (Amg), dorsal and ventral hippocampal formation (dHiFo and vHiFo). Both ipsi (left)- and contra (right)-lateral regions were dissected, except for Hyp (Fig. A. A1 a–d). Tissue blocks including LC and vPAG/DRN were removed using tissue punches (AgnTho's AB, Lidingö, Sweden, Fig. A. A1 e–f).

Total RNA was extracted using RNeasy plus mini kit (Qiagen, Dusseldorf, Germany) for the dissected regions and using RNAqueous micro kit (Life Technologies). After reverse-transcription using iScript select cDNA synthesis kit (Bio-Rad, Berkley, CA), qPCR was performed using the SYBR green PCR master mix and ABI Prism® 7000 sequence detection system (Life Technologies). Relative gene expression was determined by the $2^{-\Delta\Delta C_T}$ method and normalized to the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH, see Appendices).

2.6. Radioimmunoassay (RIA)

Both ipsi- and contralateral sides of the following regions were rapidly dissected: PFC, Amg, dHiFo, and vHiFo. The LC and vPAG were punched out as above (Fig. A. A1). The tissue samples were weighed, extracted in acetic acid, boiled, homogenized, centrifuged, lyophilized and dissolved before analysis, as previously described (Theodorsson and Rugarn, 2000).

Competitive RIA of cholecystokinin (CCK) was performed as described for galanin using antiserum Ab 2609 (Rehfeld, 1987).

2.7. Statistical analyses

GraphPad Prism version 6 (GraphPad Software, CA) was used to perform all statistical analyses using ANOVA followed up by Tukey–Kramer Multiple Comparison Test. No differences were found between the ipsi- and contra-lateral regions, hence the groups were collapsed in all