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Special Issue on Galanin

One for all or one for one: does co-transmission unify the concept of a brain galanin "system" or clarify any consistent role in anxiety?

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Abstract

Galanin (GAL) is a potential target for novel antidepressant or anti-anxiety drug development. However, no integrated role for a "brain galanin system" in anxiety has yet emerged. It is possible that such a function may be revealed by examining the interaction of GAL with norepinephrine (NE), with which it is prominently co-localized. We showed previously that enhancing stress-activation of the NE system by yohimbine (YOH) pretreatment induced the release of GAL in central amygdala (CeA) to exert an anxiolytic effect on the elevated plus-maze. However, it remained to be demonstrated conclusively that GAL was co-released from NE terminals in CeA in this context, or if a multi-synaptic circuit activated GAL release from another afferent to CeA, or from local GAL neurons in the vicinity of CeA. In studies presented at the Third International Symposium on Galanin and Its Receptors, we utilized a combination of behavioral pharmacological approaches, testing the effects of YOH on the behavioral response to stress on the plus-maze after lesioning NE afferents to CeA with 6-OHDA, and anatomical approaches to identify GAL afferents to CeA that are activated in the context of stress with yohimbine pretreatment, to address these alternatives. Our results suggest that GAL was not co-released from noradrenergic terminals innervating CeA to exert an anxiolytic influence when noradrenergic activation was amplified by yohimbine pretreatment. Rather, it most likely originated from GAL neurons immediately adjacent to CeA that were activated by a non-noradrenergic afferent arising from elsewhere in the brain, itself activated by increasing NE activity. Thus, any role for brain GAL in anxiety remains region-specific, pathway specific, response specific and context-specific, which is likely to continue to present challenges to the development of novel agents targeting brain GAL for treatment of depression or anxiety.

Keywords: Anxiety; Central amygdala; Elevated plus-maze; Galanin; Norepinephrine; Yohimbine

Galanin (GAL) has been implicated in many behavioral processes, including anxiety, and thus represents a potential target for novel strategies aimed at pharmacological treatment of depression and anxiety disorders. For such strategies involving systemic drug treatment to be viable, it would be useful to be able to define an integrated role for a "brain galanin system" in the modulation of anxiety. However, no clear or consistent role for GAL at a systems level has yet emerged. Anxiolytic effects, anxiogenic effects and no effects have all been ascribed to GAL, depending on the site and route of administration, the behavioral responses studied, the contexts in which they were studied, whether agonist or antagonist drugs were used to assess GAL effects, and whether measures were made of baseline behavioral changes or changes evoked by an acute anxiety-provoking stimulus (see Khoshbouei et al., 2002a,b).

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We have observed and described some of this diversity of effect in our own past and present work. For instance, bilateral administration of the GAL antagonist M40 by local microinjection into the lateral bed nucleus of the stria terminalis (BSTL) immediately prior to 5 min immobilization stress attenuated the stress-induced reduction in open-arm exploration on the elevated plus-maze and stress-induced reduction in social behavior on the social interaction test, both measures of anxiety-like behavioral reactivity to stress (Khoshbouei et al., 2002a; Fig. 1). By contrast, M40 had no effect on baseline behavior in either test in the absence of stress. This would indicate that stress-induced release of GAL in the BSTL facilitates anxiety-like behavioral reactivity, similar to the effect we have also demonstrated for norepinephrine in this same region (Cecchi et al., 2002). However, the behavioral response to stress on both of these tests represents an inhibition of ongoing behavior. Thus, to get a better understanding of the nature of the modulatory effect of GAL, we have also examined effects of M40 administration on a response that represents an activation of behavior, shock-probe defensive burying. In this study, presented in an abstract at the Third International Galanin Symposium (Echevarria et al., 2004), we found that administration of M40 into the lateral septum, a region known to be important in the defensive burying response (Treit et al., 1993), attenuated burying behavior in a dose-dependent manner. Thus, endogenous GAL facilitated both inhibitory and activated anxiety-like behavioral responses to acute stress. However, this does not necessarily imply that GAL release causes anxiety, as we have also shown in a study presented at the 2004 Society for Neuroscience meeting, that defensive burying is an adaptive coping behavior, serving to reduce the level of stress induced by the shock-probe encounter, as active burying reduced ACTH secretion compared to that seen in rats that were not allowed to bury (Petre et al., 2004).

Further, in a previous study, we also wished to examine the interaction of GAL and norepinephrine, with which it is extensively co-localized, when they are explicitly released as co-transmitters. We pretreated animals with yohimbine, an α_2-adrenergic autoreceptor antagonist, prior to acute stress in order to amplify the activation of noraderenergic activity and recruit the co-release of GAL in the central amygdala (CeA; Khoshbouei et al., 2002b; Fig. 2). With yohimbine pretreatment, we observed a paradoxical anxiolytic reversal of the stress-induced reduction in open arm exploration on the plus-maze, and this anxiolytic effect was blocked by M40 administration into CeA (Khoshbouei et al., 2002b; Fig. 3). Thus, under conditions of heightened noradrenergic activation, GAL release is recruited in CeA to exert an anxiolytic effect, buffering the facilitatory influence of norepinephrine. In support of this conclusion, a similar anxiolytic effect following yohimbine treatment has also been observed in transgenic mice overexpressing GAL specifically in noradrenergic neurons (Holmes et al., 2002).

However, it remained necessary for us to demonstrate conclusively that the GAL released in CeA in this context was indeed the result of co-release from noradrenergic terminals. It was also possible that yohimbine, by elevating norepinephrine release elsewhere in the brain, may have activated an extra-amygdalar galaninergic afferent to CeA. Alternatively, either the noradrenergic input to CeA, or another afferent to CeA activated by norepinephrine, could have stimulated GAL release from local neurons residing in the vicinity of CeA itself. Our second abstract at the Galanin Symposium describes the results of recent experiments designed to test these alternatives (Barrera et al., 2004). First, we selec-

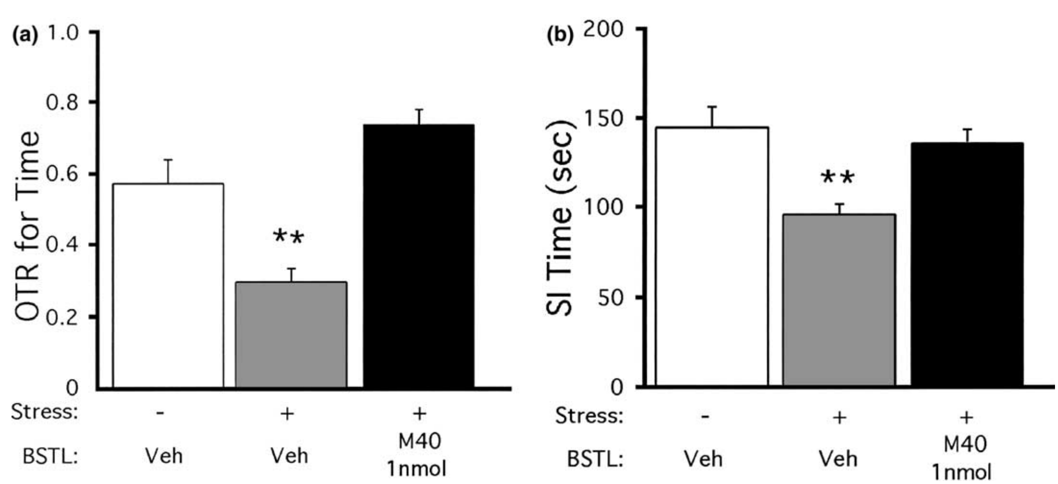

Fig. 1. Bilateral administration of M40 (0.2–1.0 nmol) into BSTL attenuated the anxiety-like reductions in open arm exploration on the elevated plus-maze (a) and social interaction time (b) induced by acute immobilization stress. In the absence of stress, M40 had no effect. Values represent mean ± SEM (n = 5–9). $^{**}p < 0.01$ compared to unstressed vehicle controls; OTR: open-to-total ratio for time. Modified and reproduced with permission from Nature Publishing Group, from Khoshbouei et al. (2002a).

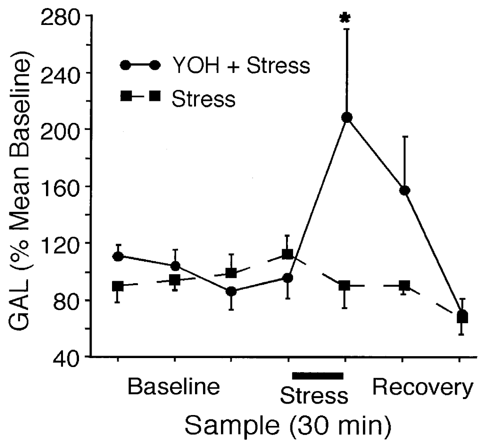

Fig. 2. Using microdialysis, an increase in GAL release was detected in CeA during acute immobilization stress (bar) only after yohimbine pretreatment. Values expressed as mean percent baseline ± SEM ($n = 7$ per group). $^*p < 0.05$ by repeated measures over time. Reproduced with permission from Elsevier, from Khoshbouei et al. (2002b).

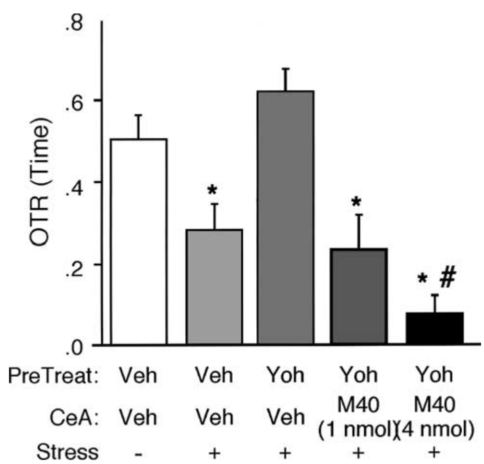

Fig. 3. Local microinjection of M40 (1.0–4.0 nmol) into CeA blocked the anxiolytic effect on the plus maze of yohimbine pretreatment before stress. Values expressed as mean ± SEM ($n = 5$–11 per group). $^*p < 0.05$ compared to both unstressed vehicle-pretreated and YOH-pretreated + stress group; $\#p < 0.05$ compared to the vehicle-pretreated stress-only group. Modified and reproduced with permission from Elsevier, from Khoshbouei et al. (2002b).

tively lesioned the noradrenergic innervation of CeA by microinjecting 6-hydroxydopamine one week prior to testing, protecting dopaminergic and serotonergic terminals with reuptake blockers, resulting in a 70% decrease in noradrenergic terminal density in CeA. When lesioned rats were tested on the plus-maze, no difference in baseline behavior was observed, although the stress-induced reduction in open-arm exploration was attenuated. However, there was no change in the anxiolytic reversal of the stress effect with yohimbine pretreatment, suggesting that this GAL-mediated anxiolytic effect was not dependent upon the integrity of noradrenergic terminals innervating CeA. This conclusion was then verified in a series of anatomical studies. First, we examined the induction of Fos expression in GAL neurons following stress with yohimbine pretreatment. Regions showing a high degree of Fos induction in GAL neurons included the paraventricular nucleus, locus coeruleus, and a cluster of GAL neurons located between CeA and the medial amygdala, in the intra-amygdalar BST (BSTIA). We then examined the correspondence between these regions and regions providing GAL innervation of CeA, by combining GAL in situ hybridization with retrograde tracing of cholera toxin B microinjected into CeA. GAL neurons exhibiting retrograde label were observed in BSTL, dorsal raphe, lateral- and tuberomammillary nuclei. GAL neurons in LC were not retrogradely labeled from CeA, although label was seen in the nearby parabrachial region, evidence that the tracer had reached the brainstem.

Thus, we conclude that the anxiolytic release of GAL induced in CeA by amplifying the noradrenergic response to stress with yohimbine pretreatment was not the result of co-transmission from noradrenergic terminals. Rather, it most likely originated from local GAL neurons in the BSTIA, immediately adjacent to CeA, activated by a non-noradrenergic afferent arising elsewhere in the brain, itself activated by increasing noradrenergic activity. Thus, we are still unable to ascribe a consistent and specific role for an integrated "brain galanin system" in modulating anxiety. Rather, the role of GAL in anxiety, at least for the time being, remains region-specific, pathway specific, response specific and context-specific. This is likely to continue to present challenges to efforts to develop novel therapeutic agents targeting brain GAL for the treatment of depression or anxiety.

Conflict of interest statement

None of the authors have any conflict or interest to report, financial or otherwise, that may inappropriately bias the conduct, interpretation or presentation of this work.

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