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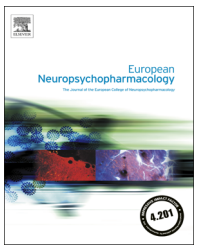

Neuropeptide Y in the central nucleus of amygdala regulates the anxiolytic effect of agmatine in rats

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Abstract
In the present study, modulation of anxiolytic action of agmatine by neuropeptide Y (NPY) in the central nucleus of amygdala (CeA) is evaluated employing Vogel's conflict test (VCT) in rats. The intra-CeA administration of agmatine (0.6 and 1.2 μmol/rat), NPY (10 and 20 pmol/rat) or NPY Y1/Y5 receptors agonist [Leu^{31}, Pro^{34}]-NPY (30 and 60 pmol/rat) significantly increased the number of punished drinking licks following 15 min of treatment. Combination treatment of subeffective dose of NPY (5 pmol/rat) or [Leu^{31}, Pro^{34}]-NPY (15 pmol/rat) and agmatine (0.3 μmol/rat) produced synergistic anxiolytic-like effect. However, intra-CeA administration of selective NPY Y1 receptor antagonist, BIBP3226 (0.25 and 0.5 mmol/rat) produced anxiogenic effect. In separate set of experiment, pretreatment with BIBP3226 (0.12 mmol/rat) reversed the anxiolytic effect of agmatine (0.6 μmol/rat). Furthermore, we evaluated the effect of intraperitoneal injection of agmatine (40 mg/kg) on NPY-immunoreactivity in the nucleus accumbens shell (AcbSh), lateral part of bed nucleus of stria terminalis (BNSTl) and CeA. While agmatine treatment significantly decreased the fibers density in BNSTl, increase was noticed in AcbSh. In addition, agmatine reduced NPY-immunoreactive cells in the AcbSh and CeA. Immunohistochemical data suggest the enhanced transmission of NPY from the AcbSh and CeA. Taken together, this study suggests that agmatine produced anxiolytic effect which might be regulated via modulation of NPYergic system particularly in the CeA.

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1. Introduction

Agmatine is a neurotransmitter abundantly localized in the brain. It is synthesized following decarboxylation of L-arginine by arginine decarboxylase in brain and peripheral tissues (Reis and Regunathan, 2000). It is metabolized to putrescine and guanido-butanoic acid by an enzyme agmatinase and diamine oxidase respectively (Reis and Regunathan, 2000). Several types of receptors are proposed as a target for the binding of agmatine through which it produced various pharmacological actions. Binding of agmatine activates both I_1/I_2 imidazoline and α_2-adrenergic receptors, while antagonizes N-methyl-*D*-aspartic acid (NMDA) receptors (Halaris and Plietz, 2007; Reis and Regunathan, 2000; Yang and Reis, 1999) and inhibits all isoforms of nitric oxide synthase enzyme (Auguet et al., 1995). Endogenously it is implicated in many psychological and physiological processes. Exogenously administered agmatine produced multiple effects on psychological phenomenon such as anxiolytic, antidepressant, anti-convulsive, memory facilitation and modulation of drug dependence (Uzbay, 2012; Kotagale et al., 2010). Furthermore, it is implicated in the physiological processes viz. nociception, neuroprotection, psychosis etc (Uzbay, 2012; Kotagale et al., 2012; Zhu et al., 2008).

Reports focusing on the distribution of agmatine demonstrated the agmatine-like immunoreactivity in several brain regions including amygdala, implicated in the regulation of anxiety-like behavior (Otake et al., 1998). Numerous studies have reported its anxiolytic profile in rodents (Gong et al., 2006; Lavinsky et al., 2003). However, the exact mechanism of its anxiolytic action has largely remained elusive.

Among the many other neuropeptides, neuropeptide Y (NPY) is upcoming and promising novel target for the management of anxiety-like behavior (Wu et al., 2011). NPY, a 36 amino acids peptide, binds to five "Y" receptor subtypes (Y1, Y2, Y4, Y5 and y6) and plays a crucial role in feeding (Daniels et al., 2001), depression (Jimenez-Vasquez et al., 2001), convulsions (Vezzani et al., 2002) and anxiety (Britton et al., 2000). Furthermore, preclinical observation supported a link between low levels of NPY and increased risk of mood and anxiety disorders in humans (Primeaux et al., 2005; Rasmusson et al., 2000). NPY is abundantly expressed in numerous brain areas including amygdala (Chronwall et al., 1985; Heilig et al., 1993). The central nucleus of amygdala (CeA) receives dense innervations of NPY from the nucleus of the solitary tract, hypothalamic arcuate nucleus (ARC) and lateral septum (Chronwall et al., 1985) and contains a high amount of NPY Y1 receptors (Wolak et al., 2003). In contrast to Y2 receptors, activation of NPY Y1 receptors in the amygdala produced dose related anxiolytic effects in rodents (Heilig et al., 1993; Sajdyk et al., 2002). Similarly, an intra-amygdala antisense oligonucleotide injection inhibits NPY Y1 receptor gene expression or mutant mice lacking NPY, generates anxiety (Bannon et al., 2000; Heilig, 1995). In addition, NPY plays overt role in agmatine induced feeding behavior (Taksande et al., 2011).

In the present study, an attempt has been made to characterize the role of NPY in CeA in mediating anxiolytic action of agmatine using VCT. In this test, thirsty animals gain water reward through a water spout but at the expense of receiving a mild electric shock delivered through metallic spout while drinking attempts. While licking in control animals was suppressed, anxiolytics reversed the suppressed behavior. Non-specific effects are assessed on non-punished water consumption. Furthermore, the effect of agmatine on endogenous NPY-containing elements in the nucleus accumbens shell (AcbSh), lateral division of bed nucleus of stria terminalis (BNSTl) and CeA was studied employing immunohistochemistry. These neuroanatomical areas contain an abundant of NPY and reported to involve in the regulation of anxiety-like behavior (Dandekar et al., 2008; Deo et al., 2010; Rangani et al., 2012).

2. Experimental procedure

2.1. Subjects

Adult male Sprague-Dawley rats (240–260 g) were group housed in acrylic cages ($24 \times 17 \times 12$ cm^3) under ambient room temperature (25 ± 2 °C) and relative humidity (50 ± 5%), maintained at 12:12 h dark–light cycle (lights on at 0700 h). Food and water were available *ad libitum* except during specific experimental protocol. All procedures employed in the present study were approved by institutional animal ethics committee and carried out under strict compliance with committee for the purpose of control and supervision of experiments on animals, Ministry of Environment and Forests, Government of India. All the behavioral studies were conducted during the light phase between 0900 and 1400 h.

2.2. Drugs

Agmatine sulfate, NPY, NPY Y1 receptors agonist [Leu^{31}, Pro^{34}]-NPY and NPY Y1 receptors antagonist BIBP3226 (N2-diphenylacetyl)-*N*-[(4-hydroxy-phenyl)-methyl]-D-arginine amide were purchased from Sigma-Aldrich Co., USA. All drugs were dissolved in artificial cerebrospinal fluid (aCSF: 140 mmol NaCl, 3.35 mmol KCl, 1.15 mmol $MgCl_2$, 1.26 mmol $CaCl_2$, 1.2 mmol Na_2HPO_4 and 0.3 mmol NaH_2PO_4, pH 7.4) containing 0.1% BSA (Taksande et al., 2011) and injected bilaterally into the CeA. Agmatine was dissolved in saline for intraperitoneal (i.p.) administration.

2.3. Intra-CeA cannulation and injections

Detail procedure of stereotaxic cannulation and post-surgical care is described in our previous study (Dandekar et al., 2008). Briefly, rats under thiopentone sodium (45 mg/kg, i.p.; Abbott Pharmaceuticals, Mumbai, India) anesthesia were implanted bilaterally by stainless steel guide cannulae (fabricated in-house) into the CeA. Stereotaxic co-ordinates, -2.56 mm posterior, ± 4.5 mm lateral to midline and -8.0 mm ventral with respect to bregma (Kokare et al., 2011; Paxinos and Watson, 1998). After appropriate wound closure, animals were housed individually and allowed to recover for 7 days. During this period, rats were injected subcutaneously (s.c.) with cefotaxime sodium (50 mg/kg; Cefantral, Lupin Pharmaceuticals, India) immediately prior to, and 2 days after, cannulation to prevent any systemic or intracranial infection. Moreover, buprenorphine (0.05 mg/kg; s.c.; Tidigesic, Sun Pharmaceuticals, India) was administered to alleviate pain following surgery. Antibiotic ointment (Neosporin-H, GlaxoSmithKline Pharmaceuticals, India) was applied locally twice daily to prevent any infection at the site of the surgery.