Neuroscience 277 (2014) 818–830

NEUROPEPTIDE Y ATTENUATES ANXIETY- AND DEPRESSION-LIKE EFFECTS OF CHOLECYSTOKININ-4 IN MICE

S. J. DESAI,[a†] C. D. BORKAR,[b†] K. T. NAKHATE,[c] N. K. SUBHEDAR[d] AND D. M. KOKARE[b*]

[a] *Department of Anatomy & Cell Biology, University of Western Ontario, Ontario, Canada*

[b] *Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur 440 033, Maharashtra, India*

[c] *Rungta College of Pharmaceutical Sciences and Research, Rungta Educational Campus, Kohka-Kurud Road, Bhilai 490 024, Chhattisgarh, India*

[d] *Indian Institute of Science Education and Research (IISER), Central Tower, Sai Trinity Building, Garware Circle, Sutarwadi, Pashan, Pune 411 021, Maharashtra, India*

Abstract—We investigated the involvement of neuropeptide Y (NPY) in the modulation of cholecystokinin-4 (CCK-4)-evoked anxiety and depression. Adult male mice were injected with vehicle, CCK-4, NPY, NPY Y1 receptor agonist [Leu^{31}, Pro^{34}]-NPY or antagonist BIBP3226, via intracerebroventricular route, and subjected to social interaction or forced swim test (FST) for the evaluation of anxiety- and depression-like phenotypes, respectively. To assess the interactions between the two systems, if any, NPYergic agents were administered prior to CCK-4 and the animals were subjected to these behavioral tests. Treatment with CCK-4 or BIBP3226 dose-dependently reduced social interaction time, while NPY or [Leu^{31}, Pro^{34}]-NPY produced opposite effect. CCK-4 treatment increased immobility time in FST. This effect was reversed by NPY and [Leu^{31}, Pro^{34}]-NPY, although BIBP3226 *per se* did not alter the immobility time. In a combination study, the anxiogenic or depressive effects of CCK-4 were attenuated by NPY or [Leu^{31}, Pro^{34}]-NPY and potentiated by BIBP3226. The brains of CCK-4 treated rats were processed for NPY immunohistochemistry. Following CCK-4 treatment, the nucleus accumbens shell (AcbSh), ventral part of lateral division of the bed nucleus of stria terminalis (BSTLV), hypothalamic paraventricular nucleus and locus coeruleus showed a reduction in NPY-immunoreactive fibers. Population of NPY-immunopositive cells was also decreased in the AcbSh, BSTLV, prefrontal cortex and hypothalamic arcuate nucleus (ARC). However, NPY-immunoreaction in the fibers of the ARC and cells of the central nucleus of amygdala was unchanged. We conclude that, inhibition of NPY signaling in the brain by CCK-4 might be causal to anxiety- and depression-like behaviors.

Key words: cholecystokinin, neuropeptide Y, anxiety, depression, immunohistochemistry.

INTRODUCTION

Neuropeptide cholecystokinin (CCK) exists in several biologically active fragments in the brain (Bradwejn, 1993; Crawley and Corwin, 1994), and acts via CCK-A and CCK-B receptors (Wank, 1995). Among those fragments, CCK-4 is abundantly expressed in the cortical and limbic structures of the brain (Durieux et al., 1988). It acts selectively through CCK-B receptors (Wank, 1995; Bradwejn and Koszycki, 2001) and is known to regulate emotional behaviors (Hinkelmann et al., 2010). CCK knock-down mice showed anxiolytic- and antidepressant-like effects (Del Boca et al., 2012). Several preclinical and clinical studies reported panicogenic and anxiogenic responses following the administration of CCK-4 (Singh et al., 1991; Bradwejn et al., 1994; Rex et al., 1994; van Megen et al., 1996; Holy and Wisniewski, 1998). In addition, treatment with CCK-B receptor antagonist elicited anxiolytic- (Singh et al., 1991; Derrien et al., 1994) and antidepressant-like responses (Hernando et al., 1994; Becker et al., 2008).

CCK-B receptors are localized on neuropeptide Y (NPY) containing neurons (Bi et al., 2004; Chen et al., 2006; Chee and Colmers, 2008); the peptide is an established orexigenic agent in the brain (Beck, 2006). NPY also exhibits potent anxiolytic and antidepressant actions via NPY Y1 receptors (Sajdyk et al., 1999, 2002; Redrobe et al., 2002). NPY showed a functional antagonism with CCK in relation to feeding behavior (Bi, 2007) and taste-bud signal transduction in the animals (Zhao et al., 2005). Similarly, chronic mild stress augmented CCK synthesis and reduced NPY levels in the hypothalamus and cerebral cortex (Kim et al., 2003). While intrahypothalamic injection of CCK caused localized reduction of NPY gene expression (Chen et al., 2008), increased NPY-immunoreactivity was noticed in the CCK-B receptors knock-out mice (Chen et al., 2006). Although these reports raise the possibility that CCK-4 and NPY may

*Corresponding author. Tel: +91-9850318502; fax: +91-712-2500355.
E-mail address: kokaredada@yahoo.com (D. M. Kokare).
† Authors contributed equally to the work.

Abbreviations: aCSF, artificial cerebrospinal fluid; AcbSh, nucleus accumbens shell; ANOVA, analysis of variance; ARC, arcuate nucleus; BSTLV, ventral part of lateral division of the bed nucleus of stria terminalis; CCK-4, cholecystokinin-4; CeA, central nucleus of amygdala; EPM, elevated plus maze; FST, forced swim test; i.c.v., intracerebroventricular; i.p., intraperitoneal; LC, locus coeruleus; NPY, neuropeptide Y; PVN, paraventricular nucleus; PFC, prefrontal cortex; PBS, phosphate-buffered saline; SEM, standard error of mean; SIT, social interaction test.

http://dx.doi.org/10.1016/j.neuroscience.2014.07.062
0306-4522/

interact centrally to regulate anxiety- and depression-like behaviors, direct evidence is lacking.

The present investigation was undertaken to define the role of NPY system in CCK-4-triggered anxiogenic- and depression-like effects. CCK-4 was administered in combination with NPYergic agents, with a view to evaluate the modulation of its anxiogenic- or depression-like effects using the social interaction test (SIT) or the forced swim test (FST) in mice, respectively. Furthermore, effect of CCK-4 treatment on the NPY-containing elements in the different brain regions like the central nucleus of amygdala (CeA), ventral part of lateral division of the bed nucleus of stria terminalis (BSTLV), nucleus accumbens shell (AcbSh), locus coeruleus (LC), prefrontal cortex (PFC), arcuate nucleus of hypothalamus (ARC) and paraventricular nucleus of hypothalamus (PVN) were evaluated by immunohistochemistry. These neuroanatomical areas were chosen since they contain an abundance of CCK binding sites and NPY neurons (Beinfeld et al., 1981; Chronwall et al., 1985; Kim et al., 2003; Deo et al., 2010), and are involved in the regulation of anxiety- (Deo et al., 2010) and depression-like behaviors (Becker et al., 2008).

EXPERIMENTAL PROCEDURES

Animals

Adult male Swiss albino mice (National Institute of Nutrition, Hyderabad, India) weighing between 25 and 30 g were used. All animals were maintained under a constant room temperature (25 ± 1 °C), relative humidity (50–70%) and on a 12:12 h light/dark cycle (lights on 07:00–19:00 h). Food and water were provided *ad libitum*. All behavioral experiments were carried out between 9:00–13:00 h under a light intensity of about 300 lux. All procedures employed in the present study were approved and carried out under strict compliance with Institutional Animal Ethics Committee, Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, MS, India.

Drugs

CCK-4 (rat; Cat. No. T6515), NPY (human; Cat. No. N5017), NPY Y1 receptor agonist [Leu^{31}, Pro^{34}]-NPY (porcine; Cat. No. N7768) or NPY Y1 receptor antagonist BIBP3226 (N2-diphenylacetyl)-N-[(4-hydroxy-phenyl)-methyl]-D-arginine amide; Cat. No. B174) were procured from Sigma, St. Louis, MO, USA and directly dissolved in the artificial cerebrospinal fluid (aCSF; Bhisikar et al., 2009). The doses of CCK-4 or NPYergic agents were selected on the basis of a preliminary dose-response study (Tables 1 and 2).

Intracerebroventricular (i.c.v.) cannulation and administrations

Detailed procedures of i.c.v. cannulation, drug administration and post-surgical care have been described earlier (Bhisikar et al., 2009; Bhorkar et al., 2014). Briefly, animals were anaesthetized with thiopentone sodium [45 mg/kg, intraperitoneal (i.p.); Abbott Pharmaceuticals, Mumbai, India], and a stainless steel guide cannula prepared in-house (Kokare et al., 2011), was implanted into the right lateral ventricle using stereotaxic coordinates, −0.8 mm posterior, +1 mm lateral to midline and 2.3 mm ventral with respect to bregma (Paxinos and Franklin, 2001). Stainless steel screws were fitted to the skull, and guide cannula was firmly attached in place using dental cement. A flush-fitting stylet was inserted into the guide cannula to prevent blockage. After cannulation, the animals were allowed to recover for 7 days. During this period, mice showing any neurological or motor deficits like impairment of locomotion, grooming or occurrence of aggressiveness, handling-induced hyper-excitability and stereotype behavior were not included in the study (Goyal et al., 2006). After the recovery period, i.c.v. injection (5 µl) was given to each mouse using a micro liter syringe (Hamilton, Nevada) connected by polyethylene tubing to an internal cannula fabricated in-house (Kokare et al., 2011), that projected 0.5 mm below the guide cannula. Before administration, the syringe and the tubing were filled with double distilled water and a small air bubble was introduced to separate the infusion solution from the double distilled water. The movement of air bubble inside the tubing confirmed the precise flow of the solution during the injection.

Fifteen minutes after i.c.v. injection, individual mice were subjected to the SIT or FST for the behavioral assessment. Studies from various laboratories show that, following i.c.v. administration, uniform dispersal of the administered agent in all brain regions occurs within 10–15 min (Ziesler et al., 1984; Yee et al., 1994; Li et al., 2006). Within a similar time span, peptides effectively alter the behavior of rodents (Kask et al., 2000; Asakawa et al., 2001; Dandekar et al., 2008; Upadhya et al., 2009). Furthermore, method of i.c.v. drug administration is standardized and routinely employed in our laboratory (Upadhya et al., 2011; Nakhate et al., 2013).

SIT for anxiety

In the first series of experiments, different groups of mice (n = 7–8/group) were injected via i.c.v. route with aCSF (5 µl/mouse), CCK-4 (50–200 ng/mouse), NPY (0.2–0.8 ng/mouse), [Leu^{31}, Pro^{34}]-NPY (0.02–0.08 ng/mouse) or BIBP3226 (0.5–2.0 µg/mouse). Fifteen minutes after drug administration, the animals were subjected to SIT for the assessment of anxiety-like behavior. Based on the results obtained in these experiments, sub-effective and effective doses were determined. In combination studies, separate groups of animals (n = 8/group) were administered i.c.v. with NPY (0.2 ng/mouse), [Leu^{31}, Pro^{34}]-NPY (0.02 ng/mouse) or BIBP3226 (0.5 µg/mouse) 15 min prior to CCK-4. After 15 min, the mice were subjected to SIT.

The SIT was performed as described by Gonzalez et al. (1998). This procedure has been standardized previously in our laboratory (Dandekar et al., 2008; Bhorkar et al., 2014). Two mice of the same gender and naïve to the test were used. The index mouse was implanted with a cannula, while the untreated partner was taken from a separate cage and placed into the center of a test box