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REGIONAL DIFFERENCES IN mu AND kappa OPIOID RECEPTOR G-PROTEIN ACTIVATION IN BRAIN IN MALE AND FEMALE PRAIRIE VOLES

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Abstract—Prairie voles are unusual mammals in that, like humans, they are capable of forming socially monogamous pair bonds, display biparental care, and engage in alloparental behaviors. Both mu and kappa opioid receptors are involved in behaviors that either establish and maintain, or result from pair bond formation in these animals. Mu and kappa opioid receptors both utilize inhibitory G-proteins in signal transduction mechanisms, however the efficacy by which these receptor subtypes stimulate G-protein signaling across the prairie vole neuraxis is not known. Utilizing [^{35}S]GTPγS autoradiography, we characterized the efficacy of G-protein stimulation in coronal sections throughout male and female prairie vole brains by [D-Ala$_2$,NMe-Phe$_4$, Gly-ol$_5$]-enkephalin (DAMGO) and U50,488H, selective mu and kappa opioid agonists, respectively. DAMGO stimulation was highest in the forebrain, similar to that found with other rodent species. U-50,488H produced greater stimulation in prairie voles than is typically seen in mice and rats, particularly in select forebrain areas. DAMGO produced higher stimulation in the core versus the shell of the nucleus accumbens (NAc) in females, while the distribution of U-50,488H stimulation was the opposite. There were no gender differences for U50,488H stimulation of G-protein activity across the regions examined, while DAMGO stimulation was greater in sections from females compared to those from males for NAc core, entopeduncular nucleus, and hippocampus. These data suggest that the kappa opioid system may be more sensitive to manipulation in prairie voles compared to mice and rats, and that female prairie voles may be more sensitive to mu agonists in select brain regions than males. © 2015 Published by Elsevier Ltd. on behalf of IBRO.

Key words: guanosine 5′-O[γ-^{35}S] triphosphate, second messenger, signaling, autoradiography, DAMGO, U50,488H, rodent, monogamy, social behavior, pair bonding.

1. INTRODUCTION

Prairie voles (*Microtus ochrogaster*) are relatively unusual among rodent species in that the male and female form lifelong, socially monogamous pair bonds after mating (Young et al., 2001; McGraw and Young, 2010; Johnson and Young, 2015). Prairie voles also, like humans, engage in alloparental behavior with both the male and female involved in rearing of the young (Young et al., 2001; Young and Wang, 2004; Ahern and Young, 2009; Ahern et al., 2011). Due to these human-like social behaviors, the neurobiology of prairie voles related to formation of pair bonds has been studied in some detail and these studies have shown that oxytocin, vasopressin, and their receptors have prominent roles in pair bond formation and maintenance (Young et al., 2001; Lim et al., 2004; Young and Wang, 2004; Donaldson et al., 2010). Additionally, both mu and kappa opioid receptors are involved in these processes. Mu opioid receptor activation in limbic forebrain regions is required for pair bond formation in female prairie voles, while kappa opioid receptor activity is required for male–male aggression following pair bond formation with a female (Burkett et al., 2011; Burkett and Young, 2012; Resendez et al., 2012, 2013). The role of opioid receptor subtypes in other brain areas in prairie vole behavior and neurobiology is an area of increased interest and investigation.

The neuroanatomical distribution of mu and kappa opioid receptor mRNA and protein has been examined in the prairie vole brain, and their distribution is reasonably comparable to that found in the rat and mouse (Inoue et al., 2013). While opioid receptor density is generally highest in brain regions that are involved in the prominent pharmacological effects of opioids in the central nervous system, the efficacy by which opioids

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Abbreviations: [^{35}S]GTPγS, guanosine 5′-O[γ-^{35}S] triphosphate; DAMGO, [D-Ala$_2$,NMe-Phe$_4$,Gly-ol$_5$]-enkephalin; EGAT, ethylene glycol tetraacetic acid); NAc, nucleus accumbens; nor-BNI, nor-binaltorphimine; PBS, phosphate-buffered saline.

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stimulate second messenger signaling through receptor activation does not always correlate with receptor density. The efficacy by which opioid agonists are able to activate second messenger systems is often determined using agonist-stimulated binding of guanosine 5′-O[γ-^{35}S] triphosphate ([^{35}S]GTPγS) binding (Sim et al., 1995). This assay has the advantage in that it can be performed autoradiographically in tissue sections, and thereby agonist efficacy can be determined throughout the neuraxis for several compounds in the same animal. Such studies are valuable in that they determine relative agonist efficacies throughout the brain for a variety of receptor subtypes efficiently, and these experiments have been used extensively to compare receptor-G-protein-coupling efficiency between animals or across pharmacological treatments (Breivogel et al., 1999; Sim-Selley et al., 2000).

In this study, we determined the efficacy of the mu opioid agonist [D-Ala$_2$,NMe-Phe$_4$,Gly-ol$_5$]-enkephalin (DAMGO) and the kappa opioid agonist U50,488H to stimulate G-protein activity using [^{35}S]GTPγS binding in striatal membranes and using autoradiography in discreet regions throughout the brain in male and female prairie voles, and confirmed receptor selectivity for each agonist at appropriate concentrations of opioid antagonists. Additionally, anatomical differences between males and females were examined for both receptor subtypes.

EXPERIMENTAL PROCEDURES

Subjects

A prairie vole colony was established at Wake Forest University Health Sciences (Winston-Salem, NC, USA) using animals imported from a colony at Emory University (Atlanta, GA, USA) originally derived from wild-caught animals from Illinois. Subjects consisted of adult, sexually naïve male (N = 8) and female (N = 8) prairie voles (age 12–18 weeks) from the Wake Forest University Health Sciences colony weighing 40–60 g at time of sacrifice. All animals used for this study were housed in same sex groups of 2–4 animals and kept on a reversed 10:14 h light:dark cycle (dark 03:00–17:00) in a temperature- and humidity-controlled vivarium within an AAALAC-approved facility. The male and female pairs were housed separately but in the same room. Female prairie voles are induced into estrous in the presence of male urine, but without direct contact with males the ovaries are quiescent and these animals had not initiated an ovarian cycle. Males were sexually naïve as well. All animals were given *ad libitum* access to high-fiber rabbit chow pellets (ProLab 5P25, LabDiet, St. Louis, MO, USA), alfalfa cubes (Country Acres Feed, Brentwood, MO, USA) and water. Bedding material consisted of ¼-inch corncob pellets (Bed-o'Cobs ¼", The Andersons Lab Bedding, Maumee, OH, USA), paper nesting material (Crink-l'Nest, The Andersons Lab Bedding) and cotton fiber nestlets (Ancare Corp., Bellmore, NY, USA). Each cage also contained one red polycarbonate tube (3-inch diameter, 6-inch length, Bio-Serv, Flemington, NJ, USA) for nesting and burrowing. All procedures were approved by the Animal Care and Use Committee of Wake Forest University (Winston-Salem, NC, USA) and were in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health (Bethesda, MD, USA).

Identification and verification of neuroanatomical brain regions

As there is currently no brain atlas available for the prairie vole, identification and verification of anatomical regions examined using the [^{35}S]GTPγS autoradiography assay was accomplished using Nissl staining at five coronal levels of prairie vole brain in conjunction with a standard rat brain stereotaxic atlas (Paxinos and Watson, 1998). For this purpose four prairie voles were anesthetized with a lethal dose of sodium pentobarbital (100 mg/kg, i.p.). A blunt 23-gauge needle connected to a 20-ml syringe by 2-mm diameter silicon tubing was inserted into the left ventricle. The tip of the needle was stabilized with a hemostat and the vole was slowly perfused with 30 ml of 0.1 M phosphate-buffered saline (PBS, pH = 7.4 at 4 °C) followed by 30 ml of 4% formaldehyde fixative solution in 0.1 M PBS. Brains were removed and post-fixed for 12 h and then transferred to 30% sucrose solution in 0.1 M PBS for cryoprotection. Serial 50-μm coronal brain sections were cut on a cryostat (Leica Microsystems, Buffalo Grove, IL, USA) and thaw-mounted on plus slides for histological processing. Nissl-stained sections were rehydrated with 0.1 M PBS for 10 min, post-fixed onto slides with 4% formaldehyde fixative for 10 min, rinsed in 0.1 M PBS and ddH_2O, stained with 1% Cresyl Violet acetate solution (Sigma–Aldrich, St. Louis, MO, USA) for 15 min at room temperature, rinsed in distilled H_2O (1 min) and dehydrated in a series of 70%, 95%, and 100% alcohol solutions. Sections were then cleared in xylene and cover slips were affixed with DPX mounting media (Sigma–Aldrich). Bright field images were captured at 20× magnification with a DS Fi2 color camera on a Nikon Eclipse Ni microscopy system (Nikon Instruments Inc, Melville, NY, USA) equipped with NIS-Elements basic research software with large image-stitching capabilities.

Agonist-stimulated [^{35}S]GTPγS binding in vole striatal membranes

For [^{35}S]GTPγS binding in membranes, striata were dissected from two female vole brains on ice and frozen in aliquots at −80 °C. Tissue samples were thawed, homogenized with a Tissumizer (Tekmar, Cincinnati OH, USA) in cold TME buffer (50 mM Tris–HCl, 3 mM $MgCl_2$, 1 mM EGTA, pH 7.4) and centrifuged at 48,000×g for 10 min at 4 °C. Pellets were resuspended in membrane buffer and centrifuged again under identical conditions. After the second centrifugation, pellets were homogenized in TME assay buffer (50 mM Tris–HCl, 3 mM $MgCl_2$, 0.2 mM EGTA, 100 mM NaCl, pH 7.7). Concentration-effect curves of agonist-stimulated [^{35}S]GTPγS binding included 0.01–10 μM DAMGO, Tocris

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