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α1b-Adrenergic Receptor Localization and Relationship to the D1-Dopamine Receptor in the Rat Nucleus Accumbens

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Abstract—The α1-adrenergic receptors (α1ARs) have been implicated in numerous actions of the brain, including attention and wakefulness. Additionally, they have been identified as contributing to disorders of the brain, such as drug addiction, and recent work has shown a role of these receptors in relapse to psychostimulants. While some functionality is known, the actual subcellular localization of the subtypes of the α1ARs remains to be elucidated. Further, their anatomical relationship to receptors for other neurotransmitters, such as dopamine (DA), remains unclear. Therefore, using immunohistochemistry and electron microscopy techniques, this study describes the subcellular localization of the α1b-adrenergic receptor (α1bAR), the subtype most tied to relapse behaviors, as well as its relationship to the D1-dopamine receptor (D1R) in both the shell and core of the rat nucleus accumbens (NAc). Overall, α1bARs were found in unmyelinated axons and axon terminals with some labeling in dendrites. In accordance with other studies of the striatum, the D1R was found mainly in dendrites and spines; therefore, colocalization of the D1R with the α1bAR was rare postsynaptically. However, in the NAc shell, when the receptors were co-expressed in the same neuronal elements there was a trend for both receptors to be found on the plasma membrane, as opposed to the intracellular compartment. This study provides valuable anatomical information about the α1bAR and its relationship to the D1R and the regulation of DA and norepinephrine (NE) neurotransmission in the brain which have been examined previously. Published by Elsevier Ltd on behalf of IBRO.

Key words: alpha1-adrenergic receptors, D1-dopamine receptors, nucleus accumbens.

INTRODUCTION

Psychostimulant abuse and addiction remain a societal problem in the United States. The latest statistics from the National Survey on Drug Use and Health indicate that slightly less than one million people over the age of 12 report having a cocaine use disorder (NSDUH, 2016). Additionally, about 11% of American children have been diagnosed with Attention Deficit Hyperactivity Disorder (ADHD), with over 70% being treated with stimulant medications (Visser et al., 2014). A problem that has arisen is the misuse of ADHD medications for nonmedical purposes, with recent surveys finding approximately 1.7 million individuals in the United States abusing stimulant drugs without a prescription (NSDUH, 2016). A goal in understanding the mechanisms behind dependency and abuse of psychostimulant drugs is to explore relevant underlying circuitry, receptors and neurochemistry in the brain.

The mesocorticolimbic pathway, norepinephrine (NE) and dopamine (DA)

The mesocorticolimbic pathway, encompassing the ventral tegmental area (VTA), nucleus accumbens (NAc) and prefrontal cortex (PFC), is interconnected structures in the brain that allow us to experience pleasure from natural rewards and from drugs that manipulate levels of two catecholamine neurotransmitters, DA and NE. The PFC controls key

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Abbreviations: ABC, avidinbiotin complex; ADHD, Attention Deficit Hyperactivity Disorder; ANOVA, analysis of variance; BSA, bovine serum albumin; D1R, D1-dopamine receptor; DA, dopamine; DAB, 3,3-diaminobenzidine tetrahydrochloride; GPCR, G protein- coupled receptor; HQ, high-quality silver enhancement; IgG, immunoglobulin G; INT, intracellular; KO, knockout animal; LC, locus coeruleus; NAc, nucleus accumbens; NE, norepinephrine; NGS, normal goat serum; PB, phosphate buffer; PBS, phosphate-buffered saline; PFC, prefrontal cortex; PMB, plasma membrane-bound; RT, room temperature; TBS, tris-buffered saline; VTA, ventral tegmental area; α1AR, alpha1-adrenergic receptor; α1bAR, alpha1b-adrenergic receptor.

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cognitive functions such as planning and impulse control, which are disrupted by disorders such as ADHD and drug addiction (Arnsten and Li, 2005; Hains and Arnsten, 2008). The NAc, divided into the core and shell, is critical for experiencing pleasurable feelings and the euphoric properties of stimulants (Wise and Bozarth, 1985, 1987; Zahm and Brog, 1992; Meredith et al., 1996). DA and NE, and their transporters and receptors, have been shown to play a role in regulating pathological changes in disorders such as ADHD and stimulant addiction (Ritz et al., 1988; Darracq et al., 1998; Pan et al., 2004; Weinshenker and Schroeder, 2007; Heal et al., 2009). For example, cocaine, amphetamine and related compounds, such as the prescription drug methylphenidate (for treating ADHD), act by increasing levels of synaptic NE and/or DA, which in turn activate the various subtypes of NE and DA receptors (Ritz et al., 1988; Ritz and Kuhar, 1989; Heal et al., 2009).

G protein-coupled receptors (GPCRs) responding to dopamine (D1Rs) and norepinephrine (α1ARs) are abundant within the mesocorticolimbic system and are essential for both the therapeutic efficacy and addictive properties of stimulants (Darracq et al., 1998; Drouin et al., 2002; Heal et al., 2009; Mitrano et al., 2012, 2014; Schmidt and Weinshenker, 2014). There are three classes of noradrenergic receptors, α1-, α2- and β-adrenergic receptors. Amongst the α1-adrenergic receptors (α1ARs), there are three subtypes, α1a, α1b and α1d (Bylund et al., 1994; Zhong and Minneman, 1999). While the subtypes of the receptor and some of their properties have been known for some time (Bylund et al., 1994; Zhong and Minneman, 1999; Chalothorn et al., 2002), their exact function and localization in various brain regions are still being elucidated. A major roadblock in understanding the functionality of each of the α1AR subtypes in the brain is the fact that subtype-specific pharmacological agents have yet to be developed (Giardina et al., 1996, 2003; Aono et al., 2015). Therefore, most studies addressing the functions of α1AR subtypes have used knockout (KO) animals (Drouin et al., 2002; Auclair et al., 2002), transfected cells (Vicentic et al., 2002), or animals that overexpress the receptors (Zuscik et al., 2000; Yun et al., 2003).

There is evidence for the α1ARs playing a role in addictive behaviors to stimulant drugs, namely in relapse or reinstatement of drug-seeking behavior (Zhang and Kosten, 2005; Weinshenker and Schroeder, 2007; Gaval-Cruz and Weinshenker, 2009; Schroeder et al., 2013). For example, the α1AR antagonist prazosin attenuates cocaine-induced reinstatement (Zhang and Kosten, 2005), while the DA β-hydroxylase inhibitor nepicastat blocks cue-induced, cocaine-induced and stress-induced reinstatement to cocaine-seeking (Schroeder et al., 2013). Using *in vivo* microdialysis in the NAc, it was shown that activation of presumed presynaptic α1ARs with the α1AR agonist methoxamine can decrease DA efflux (Saisuga et al., 2012), and when exposed to cocaine, NE release in the PFC can indirectly control DA transmission to the NAc (Drouin et al., 2002; Zhang and Kosten, 2005). Specific focus has turned to the α1bAR subtype in relation to stimulant effects and regulation of DA. For example, Drouin et al. (2002) showed α1bAR knockout mice had reduced locomotor responses to amphetamine, cocaine and morphine. Auclair et al. (2002) showed that DA-induced increases in the NAc were significantly decreased in α1bAR KO mice following amphetamine administration, as opposed to stimulating the α1bARs which resulted in increased DA-mediated locomotor responses to amphetamine (Villegier et al., 2003). These studies, just to name a few, indicate a relationship between NE, α1bARs, DA and D1Rs in the NAc in relation to neural and psychological responses to psychostimulants.

The subcellular localization of the α1AR has been characterized previously in the NAc (Mitrano et al., 2012), but localization of the specific subtype α1bAR has not been described, though suggested in some studies (Saisuga et al., 2012). If we are to truly gain an understanding of their role and relationship to DA and DA receptors, the subcellular and subsynaptic localization of the α1bAR is necessary. Additionally, even though the anatomical localization of the D1Rs has been shown in the dorsal striatum and PFC (Levey et al., 1993; Hersch et al., 1995; Dumartin et al., 1998; Mitrano et al., 2014), their relationship to the specific adrenergic receptor, α1bAR, has yet to be established in the NAc.

Therefore, this study aimed to define the subcellular and subsynaptic localization of both the α1bAR and the D1R in the core and shell of the rat NAc using immunohistochemistry and electron microscopic (EM) techniques. Next, using double labeling techniques at the EM level, the degree of finding these receptors in the same neural elements or glia was assessed. Based on previous work on the localization of the α1ARs (Mitrano et al., 2012, 2014), it was hypothesized that there would negligible colocalization of the α1bAR and the D1R. Overall, the majority of α1bARs were found presynaptically, while D1Rs were located mainly postsynaptically. Generally, minimal colocalization of these two receptors was detected in most neuronal elements as well as glial processes.

EXPERIMENTAL PROCEDURES

Animal treatment for immunohistochemistry

All procedures were approved by the Institutional Animal Care and Use Committee of Christopher Newport University. In total, 13 male adult Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA, USA; weighing approximately 200–300 g) were used for this study. Rats were anesthetized with a ketamine (100 mg/kg) and butorphanol (2 mg/kg) cocktail and transcardially perfused with 4% paraformaldehyde containing 0.1% glutaraldehyde (Electron Microscopy Sciences (EMS), Hatfield, PA, USA). Brain tissue was removed and postfixed for 24 h in 4% paraformaldehyde and cut into 60 μm sections on a vibrating microtome. Prior to immunohistochemical labeling, all tissue was exposed to 1% $NaBH_4$.

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