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ACUTE AND CHRONIC COCAINE DIFFERENTIALLY ALTER THE SUBCELLULAR DISTRIBUTION OF AMPA GluR1 SUBUNITS IN REGION-SPECIFIC NEURONS WITHIN THE MOUSE VENTRAL TEGMENTAL AREA

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Abstract—Cocaine administration increases AMPA GluR1 expression and receptor-mediated activation of the ventral tegmental area (VTA). Functionality is determined, however, by surface availability of these receptors in transmitter- and VTA-region-specific neurons, which may also be affected by cocaine. To test this hypothesis, we used electron microscopic immunolabeling of AMPA GluR1 subunits and tyrosine hydroxylase (TH), the enzyme needed for dopamine synthesis, in the cortical-associated parabrachial (PB) and in the limbic-associated paranigral (PN) VTA of adult male C57BL/6 mice receiving either a single injection (acute) or repeated escalating-doses for 14 days (chronic) of cocaine. Acute cocaine resulted in opposing VTA-region-specific changes in TH-containing dopaminergic dendrites. TH-labeled dendrites within the PB VTA showed increased cytoplasmic GluR1 immunogold particle density consistent with decreased AMPA receptor-mediated glutamatergic transmission. Conversely, TH-labeled dendrites within the PN VTA showed greater surface expression of GluR1 with increases in both synaptic and plasmalemmal GluR1 immunogold density after a single injection of cocaine. These changes diminished in both VTA subregions after chronic cocaine administration. In contrast, non-TH-containing, presumably GABAergic dendrites showed VTA-region-specific changes only after repeated cocaine administration such that synaptic GluR1 decreased in the PB, but increased in the PN VTA. Taken together, these findings provide ultrastructural evidence suggesting that chronic cocaine not only reverses the respective depression and facilitation of mesocortical (PB) and mesolimbic (PN) dopaminergic neurons elicited by acute cocaine, but also differentially affects synaptic availability of these receptors in non-dopaminergic neurons of each region. These adaptations may contribute to increased cocaine seeking/relapse and decreased reward that is reported with chronic cocaine use.

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Abbreviations: ABC, avidin-biotin complex; BSA, bovine serum albumin; LTP, long term potentiation; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; PB, parabrachial; PB VTA, parabrachial ventral tegmental area; PN, paranigral; PN VTA, paranigral ventral tegmental area; TBS, tris buffered saline; TH, tyrosine hydroxylase; VTA, ventral tegmental area.

Key words: synaptic plasticity, dopamine, tyrosine hydroxylase, ultrastructure, reward.

Dopaminergic neurons originating in the ventral tegmental area (VTA) play a critical role in the rewarding effects of cocaine and other drugs of abuse (Koob and Bloom, 1988; Le Moal and Simon, 1991). Changes in alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptor-mediated glutamatergic activation of dopaminergic neurons are thought to contribute to the development of addiction (Kalivas and Stewart, 1991; Wolf, 2003; Kessels and Malinow, 2009). Stimuli that induce long term potentiation (LTP) increase the number of functional AMPA receptors at the synapse (Liao et al., 1995; Malinow and Malenka, 2002; Park et al., 2004). Additionally, LTP can be elicited from neurons within the VTA (Bonci and Malenka, 1999) suggesting that AMPA receptor-mediated plasticity in the VTA may be important for the development of addiction to cocaine. This is supported by findings that both acute and chronic systemic cocaine administration increase AMPA-mediated glutamatergic activation of dopaminergic neurons in the VTA (Ungless et al., 2001; Saal et al., 2003; Borgland et al., 2004; Bellone and Luscher, 2006; Chen et al., 2008; but see Chen et al., 2008). Moreover, chronic cocaine administration increases both AMPA GluR1 protein expression (Fitzgerald et al., 1996) and responsiveness to local AMPA infusion (White et al., 1995; Zhang et al., 1997) demonstrating changes in AMPA receptor-mediated transmission in VTA neurons after long-term cocaine administration.

Little is known, however, regarding the effects of cocaine administration on GluR1-containing AMPA receptors in the diverse neuronal subtypes and/or subregions of the VTA (Saal et al., 2003). Functional differences in the VTA are highly dependent on anatomical connectivity and the transmitter phenotype of neurons, the majority of which are dopaminergic or GABAergic (Johnson and North, 1992). Changes in glutamate activation have been shown to affect dopamine release in both the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc) (Kalivas et al., 1989; Taber and Fibiger, 1995; Karreman et al., 1996; Meltzer et al., 1997; Kretschmer, 1999), the respective primary targets of parabrachial (PB) and paranigral (PN) projection neurons within the VTA (Carr and Sesack, 2000; Lane et al., 2008). Dopaminergic neurons of the PB VTA receive monosynaptic glutamatergic inputs from the mPFC, whereas these glutamatergic efferents target

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mainly GABAergic neurons in the PN VTA (Carr and Sesack, 2000; Sesack and Carr, 2002; Sesack et al., 2003; Geisler and Zahm, 2005). These differences in circuitry have opposing effects on dopamine release when AMPA receptor antagonist are administered into the VTA (Takahata and Moghaddam, 2000) and result in opposing behavioral output as measured by locomotor activity (Tzschentke and Schmidt, 2000a,b). Consequently, cocaine administration may produce cell type- and VTA region-specific changes in the availability and functional assembly of GluR1-containing AMPA receptors at the synapse. Moreover, these changes may differ depending on the duration of cocaine exposure. To test this hypothesis, we used electron microscopic immunogold labeling of the AMPA receptor GluR1 subunit in the PB and PN VTA regions of mice receiving either acute (single injection) or chronic (14 days of intermittent escalating-dose) systemic cocaine administration. Dopaminergic neurons were identified by immunoperoxidase labeling of tyrosine hydroxylase (TH), a mandatory enzyme for dopamine synthesis not present in other neurons of the VTA, the majority of which are GABAergic (Johnson and North, 1992). The results provide evidence that acute cocaine administration produces region-specific subcellular relocation of AMPA GluR1 subunits consistent with decreased AMPA-mediated activation of mesocortical- but increased activation of mesolimbic-projecting dopamine neurons, an effect that disappears with chronic cocaine administration. In contrast, AMPA GluR1 distributions within presumably GABAergic dendrites show little change following acute cocaine, but are more profoundly influenced by chronic cocaine administration.

EXPERIMENTAL PROCEDURES

Subjects

Twenty-one naive adult male C57/BL6 mice (Jackson Laboratories, Bar Harbor, ME, USA), weighing 19–25 g (starting weight) were individually housed and maintained on a 12-h light cycle (lights out at 6:00 PM). Food and water were available *ad libitum*. Experimental protocols involving animals followed NIH guidelines to the care and use of research animals and were approved by the Institutional Animal Care and Use Committee (IACUC) at Weill Cornell Medicall College.

Cocaine administration paradigm

Mice were randomly assigned to one of five treatment groups: Acute cocaine (n=5), chronic cocaine (n=5), single (n=3) or repeated (n=5) saline, and non-injected (n=3) controls. Acute cocaine mice received a single injection of cocaine (15 mg/kg i.p.). Chronic cocaine treated mice received three injections per day (at 10:00 AM, 4:00 PM, and 10:00 PM) for 14 consecutive days of an escalating dose paradigm of cocaine (ranging from 7.5 mg/kg to 60 mg/kg i.p. cumulative dose per day). This paradigm mimics the increasing drug intake over time as witnessed with humans addicted to cocaine (Zhang et al., 2002). Control mice were injected with saline in the same volume and intervals as mice receiving acute (one i.p. injection) or chronic cocaine (three i.p. injections per day for 14 consecutive days). In addition, to assess any changes in subcellular GluR1 related to the stress of injections, tissue from non-injected (normal) control mice were also included in the study.

Tissue preparation

All mice were deeply anesthetized with sodium pentobarbital (120 mg/kg i.p.; Sigma, St. Louis, MO, USA). For all injected animals, pentobarbital was administered 30 min after either the single (acute) or last of the chronic injections of cocaine or saline. After fully anesthetized, mice were perfused through the right ventricle with 20 ml heparin (1000 U/ml) in saline (American Pharmaceutical Partners, Schaumburg, IL, USA) followed by 50 ml of 3.75% acrolein (Polysciences, Warrington, PA, USA) in 2% paraformaldehyde in 0.1 M phosphate buffer and finally 100 ml of 2% paraformaldehyde in 0.1 M phosphate buffer (Sigma, St. Louis, MO, USA). The brains were removed from the cranium and cut into 3–4 mm coronal blocks that were postfixed in 2% paraformaldehyde for 30 min, then sliced to 40 μm sections on a Leica Vibratome VT1000 (Leica Instruments, Nussloch, Germany) in chilled 0.1 M phosphate buffer. The collected vibratome sections were placed in a storage solution (30% sucrose, 30% ethylene glycol in 0.05 M phosphate buffer, pH 7.4) at −20 °C until used for immunolabeling of GluR1 and/or TH.

Antisera

GluR1 immunolabeling was achieved using an affinity purified rabbit polyclonal antibody raised against a 13 amino acid peptide sequence corresponding to the C-terminus of rat GluR1 AMPA subunit (Chemicon, Temecula, CA, USA). The GluR1 antiserum is well characterized by immunolabeling and Western blot analysis, which shows that this antiserum recognizes a single band of 110 kDa corresponding to the GluR1 subunit with no cross-reaction with GluR2-4 subunits (Siegel et al., 1995; Aicher et al., 2002; Glass et al., 2005). To the best of our knowledge, it is unknown whether this antiserum recognizes heteromer and/or homomers of the GluR1 subunit. However, both receptor types are most likely detected by this antibody because it was derived from a small 13 amino acid peptide sequence of the C-terminus of the AMPA receptor GluR1 subunit in a portion of the tail that should not be blocked by potential conformational change associated with dimerization. The recognition of both heteromeric and homomeric forms of GluR1-containing AMPA receptors is further supported by the observed subcellular distribution of GluR1 immunogold particles in association with cytoplasmic organelles as well as synaptic and extrasynaptic portions of the plasma membrane (Lane et al., 2008), neuronal regions with proposed different AMPA receptor subunit compositions (Bellone and Luscher, 2006; Mameli et al., 2007; Argilli et al., 2008).

A mouse monoclonal antibody raised against the rate-limiting enzyme, TH, was commercially obtained from Immunostar (Hudson, WI, USA). The TH immunogen was purified to homogeneity from PC12 cells of rat origin. Western blot analysis shows that this antiserum identifies a single 60 kDa band exclusively in TH transfected, but not non-transfected, HEK293 cells (Immunostar; Lessard et al., 2010). Moreover, in brainstem catecholaminergic cell groups the TH-immunoreactivity seen using this antibody is comparable to the distribution of TH mRNA as seen by *in situ* hybridization (Rusnak and Gainer, 2005).

Immunocytochemistry

Coronal sections of tissue containing the VTA of cocaine and saline injected animals were co-processed using a dual immunogold-silver method for the detection of the antiserum against GluR1 subunit of the AMPA glutamate receptor and immunoperoxidase for detection of TH antibody. Co-processing eliminated potential variability in labeling between experimental groups. The pre-embedding dual-labeling protocol used in the present study was adapted from that of Chan and colleagues (Chan et al., 1990).

In preparation for immunolabeling, sections of tissue containing the VTA were placed in 1% sodium borohydride in 0.1 M