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DOPAMINE AND CYCLIC-AMP REGULATED PHOSPHOPROTEIN-32–DEPENDENT MODULATION OF PREFRONTAL CORTICAL INPUT AND INTERCELLULAR COUPLING IN MOUSE ACCUMBENS SPINY AND ASPINY NEURONS

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Abstract—The roles of dopamine and cyclic-AMP regulated phosphoprotein-32 (DARPP-32) in mediating dopamine (DA)-dependent modulation of corticoaccumbens transmission and intercellular coupling were examined in mouse accumbens (NAC) neurons by both intracellular sharp electrode and whole cell recordings. In wild-type (WT) mice bath application of the D2-like agonist quinpirole resulted in 73% coupling incidence in NAC spiny neurons, compared with baseline (9%), whereas quinpirole failed to affect the basal coupling (24%) in slices from DARPP-32 knockout (KO) mice. Thus, D2 stimulation attenuated DARPP-32-mediated suppression of coupling in WT spiny neurons, but this modulation was absent in KO mice. Further, whole cell recordings revealed that quinpirole reversibly decreased the amplitude of cortical-evoked excitatory postsynaptic potentials (EPSPs) in spiny neurons of WT mice, but this reduction was markedly attenuated in KO mice. Bath application of the D1/D5 agonist SKF 38393 did not alter evoked EPSP amplitude in WT or KO spiny neurons. Therefore, DA D2 receptor regulation of both cortical synaptic (chemical) and local non-synaptic (dye coupling) communications in NAC spiny neurons is critically dependent on intracellular DARPP-32 cascades. Conversely, in fast-spiking interneurons, blockade of D1/D5 receptors produced a substantial decrease in EPSP amplitude in WT, but not in KO mice. Lastly, in putative cholinergic interneurons, cortical-evoked disynaptic inhibitory potentials (IPSPs) were attenuated by D2-like receptor stimulation in WT but not KO slices. These data indicate that DARPP-32 plays a central role in 1) modulating intercellular coupling, 2) cortical excitatory drive of spiny and aspiny GABAergic neurons, and 3) local feedforward inhibitory drive of cholinergic-like interneurons within accumbens circuits. Published by Elsevier Ltd on behalf of IBRO.

Key words: excitatory and inhibitory synaptic transmission, non-synaptic gap junction–mediated morphological coupling, DA D1 and D2 receptor, mouse accumbens neurons, whole cell recording, sharp-electrode recording.

Altered glutamate transmission from the prefrontal cortex (PFC) to the ventral striatum is often implicated in schizophrenia and drug addiction; disorders that involve dopamine (DA) dysfunctional states (Grace, 1991, 2000; Greengard et al., 1999; Greengard, 2001; Albert et al., 2002; Saal et al., 2003). DA acting on D2-like receptors present on prefrontal cortical terminals (Filoux et al., 1988; Levey et al., 1993; Sesack et al., 1994; Delle Donne et al., 1997; Jansson et al., 1998) plays an important role in regulating cortico-striatal/accumbal glutamate transmission in spiny projection neurons (Brown and Arbuthnott, 1983; O'Donnell and Grace, 1994; Flores-Hernandez et al., 1997; Cepeda et al., 2001; Bamford et al., 2004). One molecule involved in DA transmission, dopamine and cyclic-AMP regulated phosphoprotein-32 (DARPP-32), exerts complex actions on DA-dependent modulation in limbic prefrontal corticoaccumbens circuits (Greengard et al., 1999) and is decreased in brains of schizophrenia patients (Albert et al., 2002).

DARPP-32 is mainly expressed in striatal/accumbens spiny projection neurons, as well as in cortical pyramidal neurons (Berger et al., 1990; Gaspar et al., 1995; Ouimet et al., 1998; Albert et al., 2002; Nairn et al., 2004; Wang et al., 2004), which are densely innervated by DA and contain DA D1- and D2-like receptor subtypes. Activation of DA D1-like receptors (D1 and D5) is reported to increase DARPP-32 phosphorylation in those neurons that express DARPP-32 proteins (Nishi et al., 1999). Studies in DARPP-32 knockout (KO) mice show that many of the physiological and behavioral effects of DA are mediated by DARPP-32 intracellular signaling pathways (Fienberg et al., 1998; Svenningsson et al., 2000, 2005). Electrophysiological responses to the DA D1/D5 agonist, including modulation of membrane excitability (Onn et al., 2003), long term potentiation and depression (Calabresi et al., 2000) and NMDA or GABAa postsynaptic currents (Flores-Hernandez et al., 2000, 2002), are absent in striatal/accumbens spiny neurons of DARPP-32 KO mice. Despite its established relationship with D1/D5 receptor-mediated mechanisms, DARPP-32 intracellular cascades are also obligatory for D2 receptor-mediated effects (Greengard et al., 1999; Nishi et al., 1999). Activation of D2-like receptors is reported to decrease DARPP-32 phosphorylation not only in the predominantly D2-containing striatopallidal projection neurons, but also in D1-containing striatonigral projection

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Abbreviations: APV, D-(−)-2-amino-5-phosphonovaleric acid; CNQX, 6-cyano-2,3-dihydroxy-7-nitro-quinoxaline disodium salt; DA, dopamine; DARPP-32, dopamine and cyclic-AMP regulated phosphoprotein-32; DMSO, dimethyl sulfoxide; EPSP, excitatory postsynaptic potential; FS, fast-spiking; IPSP, inhibitory postsynaptic potential; KO, knockout; NAC, nucleus accumbens; PFC, prefrontal cortex; WT, wild type.

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neurons (Lindskog et al., 1999). We have previously shown that DARPP-32 molecules are critical for synergistic excitation through postsynaptic D1–D2 receptor interaction in mouse striatal spiny projection neurons (Onn et al., 2003). This D1–D2 synergy in spiny neurons may be due to a direct drug action on DA receptor-coupled G-proteins (Hopf et al., 2003) and/or circuit interactions, i.e. DA-dependent modulation of other transmitter (e.g. glutamate) release within the accumbens (for review, see Nicola et al., 2000; Onn et al., 2000; Grace, 2002; Tepper et al., 2004; Surmeier et al., 2007; Wilson, 2007).

DA can also affect network interactions among neurons via actions on gap junctions and has been shown to occur within the rat striatum/accumbens (Cepeda et al., 1989; O'Donnell and Grace, 1993; Onn and Grace, 1994, 1995, 1999, 2000). Striatal/accumbens spiny neurons express the neuronal gap junction proteins connexin 32 and 36 (Micevych and Abelson, 1991; Rash et al., 2001; Meier et al., 2002; Venance et al., 2004). DA transmission via D1-like receptors is known to uncouple neurons through cAMP-dependent phosphorylation of specific connexins (Lasater, 1987; Rorig et al., 1995). We have demonstrated a DA D2 receptor dependent modulation (enhancement) of this non-synaptic, electrotonic, transmission in striatal/accumbens spiny projection neurons in anesthetized rats (Onn and Grace, 1994) and in rat brain slices (O'Donnell and Grace, 1993). This coupling is potently modulated by behaviorally (Moore and Grace, 2002) and psychiatrically relevant treatment regimens in intact animals, including amphetamine withdrawal (Onn and Grace, 2000), DA depletion (Onn and Grace, 1999) and antipsychotic drug administration (Onn and Grace, 1995). Given the potent modulation of coupling induced in intact anesthetized rats by psychiatrically relevant manipulations of the DA system, it is likely that modulation of gap junctional conductance plays a major role in the response of the mesocorticolimbic systems to decompensation, as occurs with drug dependency and schizophrenia (Grace, 1991; Greengard et al., 1999; Hyman and Malenka, 2001; Albert et al., 2002; Saal et al., 2003; Svenningsson et al., 2005).

In the present study we evaluated the incidence of intercellular coupling in wild type (WT) and DARPP-32 KO accumbens core spiny neurons and its modulation by DA D1- and D2-like agonists using conventional intracellular sharp-electrode recording in brain slices from WT and DARPP-32 KO mice. Given that we are examining the impact of altering second and third messenger systems on cellular coupling, whole-cell current-clamp recordings, in which dialysis of essential constituents from the soma may occur, were employed to compare the results obtained by intracellular sharp electrode recordings. Secondly, cortically-evoked synaptic responses were examined in accumbens neurons of WT and KO mouse slices. The effects of DA D1- and D2-like receptor agonists and antagonists were examined in identified NAC spiny neurons and aspiny interneurons with fast-spiking (FS) or cholinergic-like characteristics, which have been well characterized in the dorsal striatum (Aosaki et al., 1998; Koos and Tepper, 1999; Pisani et al., 2000; Bracci et al., 2002; Suzuki et al., 2001; Centonze et al., 2003; Tepper et al., 2004) but have not yet been studied in the NAC of rats or mice. Portions of these data were presented in abstract form (Onn et al., 2006b).

EXPERIMENTAL PROCEDURES

Animals and *in vitro* brain slice preparations

Adult male mice lacking the DARPP-32 gene were generated via gene-targeting in embryonic stem cells using standard techniques. WT and mutant mice were generated by interbreeding of heterozygotes and were gene typed by southern analysis. The detailed protocol of producing such mutant mice is described elsewhere (Fienberg et al., 1998). All mice used in this study were handled in accordance with the USPHS Publication Guide for the Care and Use of Laboratory Animals, and the specific experimental protocols were approved by the Institutional Animal Care and Use committee of the Drexel University Medical College and the University of Pittsburgh. All efforts were made to minimize the number of animals used and their suffering.

Adult male mice (3–6 months of age) were deeply anesthetized with 8% chloral hydrate (400 mg/kg; i.p.) before they were decapitated and the brain was rapidly removed, blocked and attached onto the chamber of a DSK vibratome (Ted Pella, Japan) that was filled with chilled physiological saline saturated with 95%:5% O_2:CO_2; pH=7.23. Tissue slices (350 μm in thickness) were cut in a horizontal (oblique) plane to preserve corticoaccumbens connections (Charara and Grace, 2003; Onn et al., 2003) and immediately placed into incubation vials containing the oxygenated physiological saline solution at 34 °C for at least 1 h before intracellular recording. Recordings were performed in slices superfused with continuously oxygenated physiological saline at a flow rate of ~1 ml/min under the control of a peristaltic pump. The physiological saline solutions contained (in mM): 128 NaCl, 3 KCl, 2 $CaCl_2$, 2 $MgSO_4$, 24 $NaHCO_3$, 1.25 NaH_2PO_4, and 10 D-glucose.

Intracellular sharp-electrode recordings and labeling

Sharp electrodes were pulled from 1 mm o.d. Omegadot (WPI, Sarasota, FL, USA) borosilicate glass tubing using a Flaming-Brown P-80/PC electrode puller and filled with 2% biocytin (dissolved in 3 M potassium acetate, average electrode impedance ~110 MΩ as measured *in situ*). Intracellular recordings were performed using a NeuroData 383 intracellular preamplifier, with current injected across a bridge circuit integral to the preamplifier. All membrane potential values were adjusted by subtracting the tip potentials measured at the time point when electrodes were pulled out of the cells at the termination of recording. The biocytin-filled microelectrodes were lowered into the accumbens core region under visual control using a stereomicroscope (Nikon SMZ-2B; Melville, NY, USA) by reference to anatomical landmarks on a mouse brain atlas. After impalement of a cell with stable membrane potentials, cells were injected with biocytin using 0.5–1 nA depolarizing current pulses delivered at 3.3 Hz (Onn et al., 2003, 2006a). Only one cell per slice was injected to ensure an accurate correlation between pharmacological responses and cell morphology and location.

In vitro whole cell recordings and labeling

Whole cell patch electrodes (5–7 MΩ) were pulled from 1.5 mm glass pipettes (WPI). Whole cell recordings were performed in a submersion chamber maintained at 31–32 °C using patch pipettes filled with (in mM): 115 K-gluconate, 10 Hepes, 2 $MgCl_2$, 2 KCl, 2 Mg-ATP, 2 Na_2-ATP, 0.3 GTP (pH 7.3; osmolarity: 295 mOsm) containing 0.5% biocytin (Onn et al., 2006a). Cells were visualized using an Olympus BX50WI (40× infrared lens) under an infrared