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Dopamine D2 receptor desensitization by dopamine or corticotropin releasing factor in ventral tegmental area neurons is associated with increased glutamate release

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ABSTRACT

Neurons of the ventral tegmental area (VTA) are the source of dopaminergic (DAergic) input to important brain regions related to addiction. Prolonged exposure of these VTA neurons to moderate concentrations of dopamine (DA) causes a time-dependent decrease in DA-induced inhibition, a complex desensitization called DA inhibition reversal (DIR). DIR is mediated by conventional protein kinase C (cPKC) through concurrent stimulation of D2 and D1-like DA receptors, or by D2 stimulation concurrent with activation of some Gq-linked receptors. Corticotropin releasing factor (CRF) acts via Gq, and can modulate glutamater neurotransmission in the VTA. In the present study, we used brain slice electrophysiology to characterize the interaction of DA, glutamate antagonists, and CRF agonists in the induction and maintenance of DIR in the VTA. Glutamate receptor antagonists blocked induction but not maintenance of DIR. Putative blockers of neurotransmitter release and store-operated calcium channels blocked and reversed DIR. CRF and the CRF agonist urocortin reversed inhibition produced by the D2 agonist quinpirole, consistent with our earlier work indicating that Gq activation reverses quinpirole-mediated inhibition. In whole cell recordings, the combination of urocortin and quinpirole, but not either agent alone, increased spontaneous excitatory postsynaptic currents (sEPSCs) in VTA neurons. Likewise, the combination of a D1-like receptor agonist and quinpirole, but not either agent alone, increased sEPSCs in VTA neurons. In summary, desensitization of D2 receptors induced by dopamine or CRF on DAergic VTA neurons is associated with increased glutamatergic signaling in the VTA.

1. Introduction

Several regions of the mesocorticolimbic system receive dopaminergic (DAergic) input from the ventral tegmental area (VTA), including the nucleus accumbens, prefrontal cortex, and amygdala (Koob, 2003; Oades and Halliday, 1987). Activity of the VTA is increased by salient and motivational stimuli, and the resulting DAergic output is important for the rewarding and reinforcing effect of numerous drugs of abuse (Di Chiara and Imperato, 1988; Wise, 1996; Mirenowicz and Schultz, 1996). Mesencephalic DAergic neurons possess D2 receptors that act as autoreceptors to inhibit spontaneous firing when activated by extracellular dopamine (DA) (Aghajanian and Bunney, 1977). While short-term increases in DA concentration reduce the excitability of DAergic neurons of the VTA, long-term increases of DA concentration result in desensitization of the D2 receptor to DA (Nimitvilai and Brodie, 2010). In addition, other receptors could also be affected by long-term

Non-Standard abbreviations: pDAergic, putative dopaminergic; DAergic, dopaminergic; VTA, ventral tegmental area; DA, dopamine; aCSF, artificial cerebrospinal fluid; DL-AP5, DL-2-Amino-5-phosphonopentanoic acid; CNQX, 6-Cyano-7-nitroquinoxaline-2,3-dione; MCPG, (RS)-α-Methyl-4-carboxyphenylglycine; SKF96365, 1-[2-(4-Methoxyphenyl)-2-[3-(4-methoxyphenyl)propoxy]ethyl-1H-imidazole hydrochloride; CGP35348, (3-Amino)(diethoxymethyl) phosphinic acid; CGP55845A, (2S)-3-[[(1S)-1-(3,4-Dichloro)ethyl]amino-2-hydroxypropyl](phenylmethyl) phosphinic acid hydro; CRF, corticotropin releasing factor; ASG-30, antisauvagine-30; NBI27914, 5-Chloro-N-(cyclopropylmethyl)-2-methyl-N-propyl-N′-(2,4,6-trichlorophenyl)-4,6-pyrimidinediamine hydrochloride.

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increases in DA; for example, elevated DA increases glutamatergic receptor expression in prefrontal cortex (Gao and Wolf, 2008; Sun et al., 2008).

DAergic neurons of the VTA possess high densities of D2 (Bouthenet et al., 1991; Sesack et al., 1994) and D5 receptors (Ciliax et al., 2000; Khan et al., 2000), but low levels of D3 receptors (Bouthenet et al., 1991; Diaz et al., 1995; Gurevich and Joyce, 1999). D1 and D4 receptors are quite sparse or are not detectable on DAergic VTA neurons (Meador-Woodruff et al., 1992; Mengod et al., 1992; Rivera et al., 2008). However, D1 receptors are present on glutamatergic terminals projecting to the VTA (Caillé et al., 1996).

Spontaneous firing of action potentials by DAergic VTA neurons is inhibited by the action of DA at D2 autoreceptors (Lacey et al., 1987; Brodie et al., 1990). Prolonged application of DA results in a time- and concentration-dependent decrease in the magnitude of DA-induced inhibition, a phenomenon called "DA inhibition reversal" or DIR (Nimitvilai and Brodie, 2010). DIR is mediated by extended (10–30 min) and concurrent stimulation of D2 and D1-like receptors and produces desensitization to D2 inhibition lasting up to 90 min (Nimitvilai and Brodie, 2010). DIR requires stimulation of phosphatidylinositol (PI) turnover, the activation of phospholipase C (PLC) and conventional protein kinase C (cPKC), and does not involve adenylyl cyclase, cAMP or protein kinase A (Nimitvilai et al., 2012a). Some, but not all, Gq-coupled receptors that stimulate the PLC and PKC pathways can mediate the reversal of D2 agonist inhibition (Nimitvilai et al., 2012d). One putative neurotransmitter that was not tested in that previous study was corticotropin releasing factor (CRF), which is coupled to Gq in some systems (Gutknecht et al., 2009) and which is present in the VTA (Tagliaferro and Morales, 2008; Wang and Morales, 2008).

The characterization of DIR has established parallels between DIR and conventional desensitization of the D2 receptor. Involvement of PKC in phosphorylation and internalization of D2 receptors has been reported in many systems such as HEK293 cells, striatal, hippocampal and VTA neurons (Nimitvilai et al., 2013; Namkung and Sibley, 2004; Bofill-Cardona et al., 2000; Thibault et al., 2011). Other actions of dopamine in the VTA may play a role in DIR. For example, increased activity of excitatory glutamatergic inputs during sustained dopamine administration could counteract ongoing D2-mediated inhibition and would appear as a reversal of inhibition.

Release of CRF in the CNS is linked to stress, and CRF release in the VTA may be a factor in reinstatement of abused substances. The role of CRF in the VTA is not fully understood, but as CRF is Gq-linked in some systems, it may have a role in DIR like other Gq-linked receptors (Nimitvilai et al., 2012d). Furthermore, any link between glutamate and DIR has not been explored. In the present study, we examine the possible contribution of glutamatergic and the CRF mechanisms in the phenomenon of DIR.

2. Methods

2.1. Animals

Male Fischer 344 (F344; adult rats, 4–6 weeks old, 90–150 g) or Wistar rats (90–195 g) used in these studies were obtained from Harlan Sprague–Dawley (Indianapolis, IN). Patch-clamp studies employed Wistar rats, and extracellular studies employed both strains of rats. All rats were treated in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and all experimental methods were approved by the Animal Care Committee of the University of Illinois at Chicago and by the Institutional Animal Care and Use Committee of the Scripps Research Institute.

2.2. Preparation of brain slices

Brain slices containing the ventral tegmental area (VTA) were prepared as previously described (Brodie et al., 1999a). Briefly, following brief isoflurane anesthesia and rapid removal of the brain, the tissue was blocked coronally to contain the VTA and substantia nigra; the cerebral cortices and a portion of the dorsal mesencephalon were removed. The tissue block was mounted in the vibratome and submerged in chilled cutting solution to cut coronal sections (400 μm). An individual slice was placed onto a mesh platform in the recording chamber and was totally submerged in artificial cerebrospinal fluid (aCSF) maintained at a flow rate of 2 ml/min; the temperature in the recording chamber was kept at 35 °C. The composition of the aCSF in these experiments was (in mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.24, $CaCl_2$ 2.4, $MgSO_4$ 1.3, $NaHCO_3$ 26, glucose 11. The composition of the cutting solution was (in mM): KCl 2.5, $CaCl_2$ 2.4, $MgSO_4$ 1.3, $NaHCO_3$ 26, glucose 11, and sucrose 220. Both solutions were saturated with 95% O_2/5% CO_2 (pH = 7.4). Equilibration time of at least one hour of recovery was allowed before electrodes were placed in the tissue.

2.3. Cell identification

The VTA was clearly visible in the fresh tissue as a gray area medial to the darker substantia nigra, and separated from the nigra by white matter. Recording electrodes were placed in the VTA under visual control. Putative DAergic (pDAergic) neurons have been shown to have distinctive electrophysiological characteristics (Grace and Bunney, 1984; Lacey et al., 1989). Only those neurons that were anatomically located within the VTA and that conformed to the criteria for pDAergic neurons established in the literature and in this laboratory (Lacey et al., 1989; Mueller and Brodie, 1989) were studied. These criteria include broad action potentials (2.5 ms or greater, measured as the width of the bi- or tri-phasic waveform at the baseline), slow spontaneous firing rate (0.5–5 Hz), and a regular interspike interval.

Cells were not tested with opiate agonists as has been done by other groups to further characterize and categorize VTA neurons (Margolis et al., 2006a; Margolis et al., 2003). Additional characterization, such as determining the neurotransmitter content or projection target of our cells of study (Margolis et al., 2008; Margolis et al., 2006b; Chieng et al., 2011), would have been difficult as we used extracellular recording to insure high quality, long duration recordings. The long-duration, low frequency action potentials which characterized the cells from which we recorded are associated with DA-sensitive, DA-containing neurons projecting to the nucleus accumbens, and DA sensitivity also is associated with DA VTA neurons projecting to prefrontal cortex (Margolis et al., 2008).

2.4. Drug administration

Drugs were added either to the aCSF or to the microelectrode filling solution (0.9% NaCl). Addition of drugs to the aCSF was performed using stock solutions 100 to 1000 times the desired final concentrations. Knowing the flow rate of the superfusion medium (2 ml/min), we used calibrated syringe pumps to infuse drugs at rates between 0.02 ml/min (1% of the aCSF flow rate) and 0.002 ml/min (0.1% of the aCSF flow rate) into the flowing medium, resulting in dilution of the stock solutions to the desired concentrations. Infusion of these concentrated solutions into the aCSF flow was performed prior to the solution flowing through tubing coils which permitted the drug solutions to mix completely with aCSF before this mixture reached the recording chamber. Final concentrations were calculated from aCSF flow rate, pump infusion rate and concentration of drug stock solution. The small volume chamber (about 300 μl) used in these studies permitted the rapid application and washout of drug solutions. Typically drugs reach equilibrium in the tissue after 2–3 min of application.

When drugs were added to the microelectrode filling solution (0.9% NaCl), a concentration about 10 times greater than the concentration used in the extracellular medium was needed. In all of our previous studies in which agonists and antagonists were delivered via the recording pipette (Nimitvilai et al., 2012a), the effective concentration of drugs was ten-fold higher than the effective concentration used in the extracellular medium. The concentrations of drugs used in the present study were likewise ten-fold higher than the concentrations reported in the literature for selective action. To allow time for the drug to diffuse from the pipette to the cell, the effects of bath-applied drugs were tested no less than 20 min after initiating the recording; this pipette-application method has produced comparable results to the administration of drugs through the extracellular medium in the cases in which both methods were tested, with the advantage of more localized application and reduced expense. Such local delivery of drugs through recording pipettes has been used by our lab and others (Nimitvilai et al., 2012a; Pesavento et al., 2000). One disadvantage of this method is that the exact concentration of drug received by the neurons from which we recorded is unknown.

DA hydrochloride, quinpirole, and most of the salts used to prepare the extracellular media were purchased from Sigma (St. Louis, MO). DL-AP5, CNQX, MCPG, lamotrigine, riluzole, SKF96365, bicuculline, CGP35348, CRF, urocortin, antisauvagine-30, CGP55845A and NBI27914 were purchased from R &D Systems/Tocris Bioscience (Minneapolis, MN).

2.5. Extracellular recording

Extracellular recording was chosen for these studies as this method permits the recordings to be of long duration and allows us to assess the effects of extended exposure (>60 min) to drugs. The limitation of only measuring spontaneous action potential frequency (rather than membrane potential or other electrophysiological parameters) is counterbalanced by the advantage of being able to determine the time course of drug actions and interactions without disrupting the internal milieu. Extracellular recording electrodes were made from 1.5 mm diameter glass tubing

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