Neuropharmacology 103 (2016) 69–78

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Persistent elevation of D-Aspartate enhances NMDA receptor-mediated responses in mouse *substantia nigra pars compacta* dopamine neurons

Paraskevi Krashia [a], Ada Ledonne [a], Annalisa Nobili [a, b], Alberto Cordella [a, c], Francesco Errico [d, e], Alessandro Usiello [d, f], Marcello D'Amelio [a, b], Nicola Biagio Mercuri [a, c], Ezia Guatteo [a, *], Irene Carunchio [a, c]

[a] Department of Experimental Neurology, IRCCS Santa Lucia Foundation, Rome, Italy
[b] Department of Medicine, Unit of Molecular Neurosciences, University Campus-Biomedico, Rome, Italy
[c] Department of Systems Medicine, University of Rome 'Tor Vergata', Rome, Italy
[d] Ceinge Biotecnologie Avanzate, Naples, Italy
[e] Department of Molecular Medicine and Medical Biotechnology, University of Naples "Federico II", Naples, Italy
[f] Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, Second University of Naples (SUN), Caserta, Italy

ARTICLE INFO

Article history:
Received 26 May 2015
Received in revised form
11 December 2015
Accepted 14 December 2015
Available online 17 December 2015

Keywords:
D-aspartate oxidase knockout
Midbrain dopamine neuron
Substantia nigra pars compacta
Excitatory amino-acid transporter
L-Aspartate
NMDA receptor

ABSTRACT

Dopamine neurons in the *substantia nigra pars compacta* regulate not only motor but also cognitive functions. NMDA receptors play a crucial role in modulating the activity of these cells. Considering that the amino-acid D-Aspartate has been recently shown to be an endogenous NMDA receptor agonist, the aim of the present study was to examine the effects of D-Aspartate on the functional properties of nigral dopamine neurons. We compared the electrophysiological actions of D-Aspartate in control and D-aspartate oxidase gene (*Ddo*$^{-/-}$) knock-out mice that show a concomitant increase in brain D-Aspartate levels, improved synaptic plasticity and cognition. Finally, we analyzed the effects of L-Aspartate, a known dopamine neuron endogenous agonist in control and *Ddo*$^{-/-}$ mice. We show that D- and L-Aspartate excite dopamine neurons by activating NMDA, AMPA and metabotropic glutamate receptors. *Ddo* deletion did not alter the intrinsic properties or dopamine sensitivity of dopamine neurons. However, NMDA-induced currents were enhanced and membrane levels of the NMDA receptor GluN1 and GluN2A subunits were increased. Inhibition of excitatory amino-acid transporters caused a marked potentiation of D-Aspartate, but not L-Aspartate currents, in *Ddo*$^{-/-}$ neurons. This is the first study to show the actions of D-Aspartate on midbrain dopamine neurons, activating not only NMDA but also non-NMDA receptors. Our data suggest that dopamine neurons, under conditions of high D-Aspartate levels, build a protective uptake mechanism to compensate for increased NMDA receptor numbers and cell hyper-excitation, which could prevent the consequent hyper-dopaminergia in target zones that can lead to neuronal degeneration, motor and cognitive alterations.

1. Introduction

D-Aspartate (D-Asp), like its L-enantiomer (L-Aspartate; L-Asp) is an endogenous NMDA receptor ligand, binding to the glutamate site of the GluN1 and GluN2 subunits (Errico et al., 2012; Kiskin et al., 1990; Ota et al., 2012; Verdoorn and Dingledine, 1988). In addition to NMDA receptors, D-Asp can also activate $mGlu_5$ metabotropic glutamate receptors (Molinaro et al., 2010). D-Asp is present in the brain at high concentrations during embryogenesis and early life, accumulating in the soma, synaptic vesicles and

Abbreviations: aCSF, artificial cerebrospinal fluid; BMAA, β-*N*-methylamino-L-alanine; C_m, cell capacitance; D-Asp, D-Aspartate; DDO, D-aspartate oxidase; DA, dopamine; DAergic, dopaminergic; EAATs, excitatory amino-acid transporters; I_h, hyperpolarisation-activated inward current; L-Asp, L-Aspartate; MT, medial terminal nucleus; R_m, membrane resistance; SNc, *substantia nigra pars compacta*.

* Corresponding author.
E-mail address: e.guatteo@hsantalucia.it (E. Guatteo).

http://dx.doi.org/10.1016/j.neuropharm.2015.12.013
0028-3908/

synaptosomes of neurons (Dunlop et al., 1986; Neidle and Dunlop, 1990; Wolosker et al., 2000). *In vitro* studies indicate that it can be released in a Ca^{2+}-dependent manner after electrical or chemical stimulation (Muzzolini et al., 1997; Palmer and Reiter, 1994; Savage et al., 2001) and its uptake is mediated by Na^{+}-dependent excitatory amino-acid transporters (EAATs; Adachi et al., 2004; Koyama et al., 2005).

D-Asp is produced by a not-yet identified mammalian aspartate racemase (Tanaka-Hayashi et al., 2015; Kim et al., 2010) and is catabolised by D-Aspartate oxidase (DDO; Katane and Homma, 2010). In fact, the distributions of D-Asp and DDO in the brain are opposed, with high levels of one correlating with low levels for the other (Schell et al., 1997). DDO is highly expressed in the adult brain resulting in low levels of endogenous D-Asp during adulthood (Katane and Homma, 2010). Thus, a targeted deletion of the *Ddo* gene in adult mice ($Ddo^{-/-}$) determines a 10–20-fold increase of D-Asp in different brain areas such as the cortex, striatum, hippocampus, cerebellum and olfactory bulb (Errico et al., 2006, 2008a,b; 2011a,b; Huang et al., 2006). As a direct consequence of D-Asp increase, $Ddo^{-/-}$ brains display high contents of endogenous NMDA, the N-methyl derivative of D-Asp (D'Aniello et al., 2000a,b; Errico et al., 2006).

Information from $Ddo^{-/-}$ mice was crucial for understanding the physiological role of D-Asp in the brain. For example, in CA1 hippocampal pyramidal cells, young adult $Ddo^{-/-}$ mice show enhanced NMDA receptor-dependent LTP and have enhanced cognitive properties, improved memory and spatial learning. In the hippocampus and the prefrontal cortex this is accompanied by increases in spine density and dendritic length (Errico et al., 2008a, 2011b, 2012, 2014). In accordance with an abnormally high NMDA receptor transmission, $Ddo^{-/-}$ mice are also characterized by loss of the corticostriatal long-term depression (Errico et al., 2008b, 2011a, 2012). It was also reported that in $Ddo^{-/-}$ mice, increased D-Asp contents since perinatal phases counteract phencyclidine-induced schizophrenia-like behaviours and cerebral dysfunction (Errico et al., 2013, 2015), pointing to a potential role for D-Asp in the therapeutics of schizophrenia.

Dopaminergic neurons (DAergic) in the *substantia nigra pars compacta* (SNc) are the main source of dopamine (DA) in the mammalian central nervous system. DA, released from these neurons, plays an important role in the control of multiple brain functions including voluntary movement and cognition (Matsumoto and Takada, 2013; Pasquereau and Turner, 2015) and alterations in the DAergic transmission have long been associated with neurological disorders such as Parkinson's disease and schizophrenia (Howes and Kapur, 2009; Przedborski, 2005). Unlike the better-characterised role of D-Asp in the striatum and the hippocampus, target areas of DAergic neurons (Fallon, 1981; Prensa and Parent, 2001; Scatton et al., 1980), there is scarce information on the role of D-Asp in the SNc. Thus, in the present study we investigated the role of D-Asp on the functional properties of SNc DAergic neurons. We used $Ddo^{-/-}$ mice to examine whether enhanced endogenous D-Asp levels in these animals can modify glutamate receptor expression and function, compared to control littermates ($Ddo^{+/+}$). Finally, we investigated whether changes in the D-Asp content of the $Ddo^{-/-}$ brain can alter the responses of DA neurons mediated by L-Asp, an endogenous agonist in the SNc (Abarca et al., 1995).

Our results unveiled an unexpected role of D-Asp in mediating not only NMDA but also non-NMDA receptor glutamatergic transmission in the SNc. We also show that, in conditions of increased endogenous levels of D-Asp, in $Ddo^{-/-}$ mice, NMDA currents are enhanced, membrane levels of NMDA receptor subunits are increased and that DAergic neurons compensate for these alterations by an enhanced mechanism of EAAT-mediated D-Asp transport. Our data provide new insights into the physiological role of D-Asp in mediating glutamatergic transmission in the midbrain and suggest that D-Asp, by regulating the activity of DAergic neurons, could modulate DA transmission and affect motor, as well as, cognitive functions.

2. Material and methods

2.1. Animals

Animal usage adhered to ARRIVE guidelines and complied with the ethical guidelines of the European Commission Directive (2010/63/EU) and the Italian Health Ministry (Art.31, D.Lgs 26/2014; project protocol: DM55/2014-PR) and was approved by the Animal Ethics Committee of the Fondazione Santa Lucia. $Ddo^{+/+}$ and $Ddo^{-/-}$ mice (C57BL/6 background, P17–P23 or adults) were housed in a temperature- and humidity-controlled environment (free access to food and water; 12 h dark/light cycle). All efforts were made to minimize animal suffering and reduce the total number of animals used. A total of 55 animals (males and females) were used.

2.2. Acute brain slice preparation

Horizontal midbrain slices (230–300 μm; Leica VT1200S vibratome) were obtained following isoflurane or chloral hydrate anaesthesia (400 $mg.kg^{-1}$, i.p.) and quick decapitation. Brains were removed and placed in chilled bubbled (95% O_2, 5% CO_2) artificial cerebrospinal fluid (aCSF) containing (mM): NaCl 126, $NaHCO_3$ 24, glucose 10, KCl 2.5, $CaCl_2$ 2.4, NaH_2PO_4 1.2, $MgCl_2$ 1.2 (pH 7.4; 290 $mOsm.L^{-1}$). Slices were hemisectioned and incubated in aCSF (32–33 °C) for at least 40 min prior to usage.

2.3. Electrophysiological recordings

Slices chosen for recordings were transferred to the recording chamber (0.6 mL) of an upright microscope (Axioskop 2-FS; Zeiss) and were continuously perfused with aCSF (32–33 °C). Whole-cell recordings were conducted from P17–P23 mice. SNc DAergic neurons, visualized using infrared differential interference contrast, were identified as tightly packed, medium-sized cells adjacent to the medial terminal nucleus (MT). Whole-cell currents (−60 mV, with a MultiClamp 700B) or spontaneous cell firing (in current-clamp, I = 0 mode) were filtered at 1–4 kHz using the amplifier's in-built low-pass filter, digitised with Digidata 1322A and computer-saved using Clampex 9 (all from Molecular Devices) at a sampling rate of 4-times the filter frequency. Electrodes (3–5 MΩ), pulled from thin-wall filamented glass (World Precision Instruments) were filled with a solution containing (mM): 135 K-gluconate, 10 KCl, 10 HEPES, 2 $MgCl_2$, 4 ATP-Mg^{2+}, 0.3 GTP-Na^{+}, 0.1 EGTA, 0.045 $CaCl_2$ (pH 7.3, 280–290 $mOsm.L^{-1}$). DAergic neurons were further identified by the expression of a prominent hyperpolarisation-activated inward current (I_h) in response to hyperpolarizing voltage steps (from −60 mV to −120 mV at −20 mV intervals, 1 s), slow spontaneous firing (0.5–4 Hz) and a transient outward current after a brief application of dopamine (30 μM, 25–60 s) in voltage-clamp or after hyperpolarisation induced by quinpirole (1 μM) in current-clamp. NMDA (bath-applied; 20 or 50 μM) currents were recorded with or without Mg^{2+} in the pipette and aCSF solution, in the presence of bath-applied glycine (50 μM), picrotoxin (100 μM), NBQX (10 μM) and lidocaine (200 μM). Recordings with access resistance higher than 20 MΩ were discarded. No liquid junction potential correction was applied.

Extracellular recordings (non-visual) from adult mice (older than 2 months) were performed by slowly moving the recording