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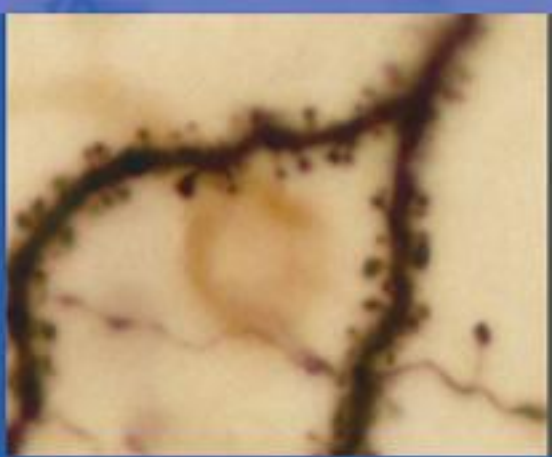
The Basal Ganglia IX



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Nitric Oxide Modulation of the Dopaminergic Nigrostriatal System: Focus on Nicotine Action

Vincenzo Di Matteo, Massimo Pierucci, Arcangelo Benigno, Ennio Esposito, Giuseppe Crescimanno, Maurizio Casarrubea, and Giuseppe Di Giovanni

Abstract Nitric oxide (NO) signalling plays an important role in the integration of information processed by the basal ganglia nuclei. Accordingly, considerable evidence has emerged indicating a role for NO in pathophysiological conditions such as Parkinson's disease (PD), schizophrenia and drug addiction. To further investigate the NO modulation of dopaminergic function in the basal ganglia circuitry, in this study we used in vivo electrophysiology and microdialysis in freely-moving rats. Pharmacological manipulation of the NO system did not cause any significant changes either in the basal firing rate and bursting activity of the dopamine (DA) neurons in the substantia nigra pars compacta (SNc) or in DA release in the striatum. In contrast, the disruption of endogenous NO tone was able to counteract the phasic dopaminergic activation induced by nicotine treatment in both experimental approaches. These results further support the possibility that nicotine acts via a NO mechanism and suggest a possible state-dependent facilitatory control of NO on the nigrostriatal DA pathway. Thus, NO selectively modulates the DA exocytosis associated with increased DA function.

1 Introduction

Nitric oxide (NO) has been associated with a variety of physiological and pathological processes in the human body since it was identified as a novel signal molecule by Furchgott and Zawadzki (1980). NO is synthesized from L-arginine by a nitric oxide synthase (NOS) using nicotinamide adenine dinucleotide phosphate (NADPH) and molecular oxygen (Bian and Murad 2003). To date, three isoforms

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of NOS, that is, neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS), have been identified. While nNOS and eNOS are constitutively expressed, the expression of iNOS is induced through the inflammatory response process of cells to infections or injuries (Dawson and Dawson 1998). The characteristics of neurotransmitter NO are that (a) it is synthesized postsynaptically, (b) it is not stored in vesicles being a diffusible gas, (c) it does not act at conventional receptors on the surface of adjacent neurons and (d) it can act as a retrograde messenger diffusing to the presynaptic terminal. Based on this evidence we can affirm that NO in the nervous system works as an unorthodox neurotransmitter. A major biochemical function of NO is to activate the soluble form of guanylyl cyclase (sGC), inducing the accumulation of cyclic guanosine monophosphate (cGMP) in target cells. Cyclic-GMP subsequently acts via protein kinases, phosphodiesterases, and perhaps directly on ion channels (Arnold et al. 1977; Bredt et al. 1990). Furthermore, NO can exert its biological effects through other mechanisms, such as modulating the function of monoamine transporters and S-nitrosylation of receptors. It has been demonstrated that NO can S-nitrosylate the NMDA receptor leading to its down-regulation (Choi and Lipton 2000).

2 Nitric Oxide Distribution in the Basal Ganglia

Nitric oxide signalling plays an important role in controlling motor behaviour modulating the integration of information processed by the basal ganglia nuclei. Most likely, it interacts with dopaminergic (DAergic), serotonergic, cholinergic and glutamatergic neurotransmission at different levels of these nuclei. Consistently, mice mutants for nNOS have altered locomotor abilities, and rats and mice treated with various NOS inhibitors show problems with fine motor control. NO, furthermore, antagonizes the increase in locomotor activity found after DA agonist administration. The pharmacological blocking of nNOS decreases locomotion and induces catalepsy in different animal species [see Del Bel et al. (2007) for a review]. Although NOS neurons are present throughout all basal ganglia nuclei and in other regions involved in motor control such as the motor cortices and the pedunculopontine tegmental nucleus (PPTg), their concentration varies significantly (Bredt et al. 1990; Del Bel et al. 2007; Egberongbe et al. 1994; Eve et al. 1998; Garthwaite and Boulton 1995; Leontovich et al. 2004; Nisbet et al. 1994; Vincent and Kimura 1992).

In comparison with other brain centres, the substantia nigra (SN) may be considered a rather NOS cell-poor nucleus (Johnson and Ma 1993; González-Hernández et al. 2000). Some studies even failed to detect any NADPH-diaphorase (NADPH-d) (+) cells in the SNc (Govsa and Kayalioglu 1999). However, a double-labelling study showed that in general few small fusiform neurons (not more than 1%) express NOS on the dorsal border of the rostrolateral part of the SNc, where it touches the zona incerta pars ventralis. These cells appear to be tyrosine hydroxylase (TH)-(+) DA neurons (González-Hernández et al. 2000). By contrast, Del Bel and colleagues have revealed in the SNc a population of NOS neurons that have

almost the same density as those present in the ventral pallidum and nucleus accumbens (Del Bel and Guimarães 2000; Gomes and Del Bel 2003). The scenario appears different in the substantia nigra, pars reticulata (SNr); in fact, many NADPH-d (+) dendrites and axon-like processes are present, some of which have close relationship with vessels (Govsa and Kayalioglu 1999). The origin of these dendrites and axon-like processes may be local, constituted by an ample intrinsic subpopulation of SNr γ-aminobutyric acid (GABA)/NOS neurons, and extrinsic, constituted by medium to large cholinergic/NOS neurons of the PPTg and laterodorsal tegmental nucleus (LDTg) (Gonzáles-Hernández and Rodríguez 2000). Within the SNr two types of GABA cells co-express NOS: one is represented by large cells in the rostrolateral part of the SNr (rlSNr) containing parvalbumine (PV) and NOS (rlSNr) and another population is constituted by small GABA cells located in the rostromedial portion of the SNr (González-Hernández and Rodríguez 2000).

The NOS expression in the striatum has been more extensively studied (Bernácer et al. 2005; Egberongbe et al. 1994; Eve et al. 1998; Govsa and Kayalioglu 1999; Johannes et al. 2003; Leontovich et al. 2004; Nisbet et al. 1994; Vincent and Kimura 1992). NOS neurons are one type of the four different classes of interneurons present in the striatum, and in the human they populate the entire striatum, including the tail and head of the caudate nucleus. They represent 1–2% of all striatal cells and are spiny, co-expressing NK1 receptors, somatostatin and neuropeptide Y (Vincent and Kimura 1992). The striatal nitrergic neurons are essentially small in diameter (12–25 μm), slender, bipolar and fusiform with a long dendrite. The neuronal population of the human striatum seems to be very heterogeneous. In fact, up to 12 different subtypes of NOS neurons have been described in humans and, strikingly, one of them is a large reticular NOS cell that resembles the characteristics of a projecting neuron (Johannes et al. 2003). This efferent nitrergic cell was demonstrated to project to the insular cortex. Thus, at least some of the NOS reticular neurons of the human striatum have direct cortical projections, though the existence of their axon collaterals in striatal tissues close to the maternal cells also demonstrates that they influence surrounding cells (Leontovich et al. 2004). Moreover, the matrix is the striatal compartment with the densest NOS neuronal population that tends to be located at the boundaries between the striosomes and the matrix, as well as at the boundaries between the core and the peripheral region of the striosomes (Bernácer et al. 2005). This finding has an important functional implication, because the NOS neurons that occur at the edges between the two compartments are thought to mediate interactions between the medium spiny neurons (MSNs) of the matrix and the striosomes. Despite the recent advances, the role of NOS interneurons in the striatum is still not clear. Among others their postulated functions are (a) to control local blood flow in the striatum by releasing NO acting directly on sGC in the vascular smooth muscle and causing vasodilatation and (b) to produce NO that acts as a neurotransmitter modulating striatal discharge and plasticity, either through direct interactions with ligand-gated channels or by influencing surrounding striatal MSNs via the stimulation of second messenger systems (West and Grace 2002).

Messenger-RNA expression studies have revealed a scattered subpopulation of NOS neurons in the internal segment and medial medullary lamina (MML) of the globus pallidus (GP) and in almost all neurons of the subthalamic nucleus (STN), which are presumed to be glutamatergic and excitatory. Moreover, it is important to note that NOS neurons have not been detected in the external GP (GPe). This may have functional implications, since it is the internal segment of the GP (GPi) and not the GPe that relays the basal ganglia output to the motor nuclei of the thalamus. In the GP, NO is probably co-localized with GABA, which has previously been shown to be the neurotransmitter of virtually all pallidal neurons (Nisbet et al. 1994; Eve et al. 1998).

3 Involvement of NO in Neurodegeneration of Dopaminergic Nigrostriatal System

Nitric oxide is a Janus-faced molecule and, notwithstanding, the exact role (i.e. neuroprotective vs. neurotoxic) it plays in neurodegenerative disorders is still ambiguous. Substantial evidence demonstrates a causative role for NO in the degeneration of DAergic neurons of the nigrostriatal pathway in PD (Esposito et al. 2007; Di Matteo et al. 2006; Duncan and Heales 2005; Zhang et al. 2006; Eve et al. 1998; Nisbet et al. 1994; Hunot et al. 1996). Consistently, NOS polymorphisms increased expression of astroglial iNOS, and nNOS have been reported in PD patients and in different toxin-induced experimental models of PD. Evidence in animal models and in PD patients suggests that NOS is up-regulated in the basal ganglia nuclei and enhanced NO formation takes place after partial injury of the nigrostriatal DAergic system. The exact mechanisms of the NO contribution to neurodegenerative diseases are not completely understood. Multiple lines of evidence indicate that NO is associated with excitotoxicity, DNA damage and protein modifications, which are common pathogenic mechanisms involved in multiple neurodegenerative diseases (Del Bel et al. 2007). Nevertheless, NO produced by either nNOS or iNOS plays an important role in DA degeneration. iNOS once induced remains active for several hours to days and produces NO in 1,000-fold greater quantities than the constitutive enzyme nNOS. A robust increase in iNOS mRNA levels has been observed after lipopolysaccharide (LPS), 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP) or 6-hydroxydopamine (6-OHDA) injection in striatum and SN (Dawson and Dawson 1998; Bian and Murad 2003). Damage to striatal DAergic fibres seems to be mainly mediated by NO produced by nNOS, while the damage to nigral DAergic neurons is largely inflicted by NO generated by iNOS. Evidence from human post-mortem studies has revealed an increase of NOS mRNA expression only in MML of the GP and in dorsal STN. Instead, a reduction has been shown to occur in the striatum although it was not statistically significant (Eve et al. 1998). Such altered activity of NOS neurons of the MML and STN may play a role in the compensatory up-regulation of nigrostriatal DAergic neurotransmission in PD, but might also exert an excitotoxic effect on striatal

neurons and nigrostriatal terminals. In animal 6-OHDA-models of PD nNOS expression is reduced while a proportion of nNOS nerve fibres in the striatum is apparently lost following DAergic deafferentation, resulting in a 50% decrease in NOS activity, and depression of the NO-cGMP pathway (De Vente et al. 2000; Sancesario et al. 2004). In contrast, Gomes et al. (2003) showed that 6-OHDA lesion induced a significant increase in NOS cell numbers in the ipsilateral dorsal striatum while a decrease was seen in the ipsilateral SNc and contralateral nucleus accumbens.

4 Nitric Oxide Modulation of the Activity of Dopaminergic Nigrostriatal System

Recently, after we had investigated the role of NO in MPP^+ (Di Matteo et al. 2006) and 6-OHDA-induced nigral neurodegeneration (Di Matteo et al. 2009), we have been studying the NO modulation of the activity of the DAergic nigrostriatal system. Although there have been numerous neurochemical studies (see West et al. 2002) the manipulation of NO within the SNc has not been investigated under normal conditions using an electrophysiological approach. Indeed, only the results of modification of tonic striatal NO tone are available at the moment (West and Grace 2000). In particular, the effect of striatal NO has been investigated on the responsiveness of SNc DA neurons to the intermittent electrical stimulation of the striatum and orbital prefrontal cortex (oPFC). Increasing NO tone in the striatum was able to counteract the decrease in firing rate of DA cells observed in control animals during intermittent stimulation. Additionally, removal of NO tone increased the proportion of DA neurons responding to striatal stimulation and increased the prevalence of the initial inhibitory responses (West and Grace 2000). Thus, it has been proposed that NO may play a pivotal role in controlling the delicate homeostatic processes that normally provide stability to the DA-nigral system. Indeed, it may be capable of dynamically regulating the relative phasic DA responsivity via its action on tonic DA levels, in a manner dependent on the arousal state of the animal. Under rest, NO produced by glutamatergic activation of NOS interneurons might increase DA release either by intensifying glutamate (GLU) release or by influencing the activity of DA transporter (DAT), decreasing DA uptake and possibly causing a DA reverse release. This increase in tonic DA would down-modulate spike-dependent phasic dopamine release via stimulation of the very sensitive DA autoreceptors present on DA terminals. In contrast, during behavioural arousal, NO exerts an opposite effect on tonic extracellular DA levels that seems to be concentration-dependent. The strong production of NO, caused by intense glutamatergic corticostriatal transmission, would result in the inhibition of NMDA receptor function and produce less inhibition of phasic DA release via disinhibition of the DA autoreceptors (West et al. 2002). As striatal NO controls DA concentration mutually striatal NO is also under a DAergic influence (Sammut et al. 2006, 2007); indeed both electrical and chemical stimulation of the SNc elicited a robust

surge in striatal NO efflux. This release seems to be neuronally dependent being blocked by pre-treatment with nNOS inhibitors and also evoked only by high-frequency stimulation that resembles the natural burst firing of DA SNc neurons. This last piece of evidence indicates that NO efflux occurs only when DA transmission is phasically increased and suggests that information transmitted via the nigrostriatal pathway during DA cell burst firing may be processed and/or amplified by NOS interneurons (Sammut et al. 2006, 2007). Dopamine within the striatum could directly modulate NO efflux, exciting NOS interneurons through the activation of $DA_{1/5}$ receptors present on their somas and increasing the release of NO (Sammut et al. 2006). Alternatively, DA modulates striatal NO levels via D_2 receptors in an opposing manner. This inhibitory control seems to be indirect and it is plausible that D_2 receptors are in fact presynaptically localized on GLU and acetylcholine (ACh) fibres impinging on NOS interneurons (Sammut et al. 2007).

5 NO/DA Interaction: Focus on Nicotine Effect

5.1 Experimental Data

To further investigate NO modulation of the nigrostriatal system, in this study we used in vivo electrophysiology and microdialysis in the rat. Extracellular single-unit recordings coupled with microiontophoresis were performed from putative DAergic-containing neurons in the SNc; local DA and its metabolite dihydroxyphenylacetic acid (DOPAC) levels were studied in the striatum by in vivo microdialysis in freely-moving rats [for more technical details, see Di Giovanni et al. (1999)].

Dopaminergic and non-dopaminergic (presumably GABA-ergic) neurons in the SNc and SNr, respectively, were identified on the basis of their established anatomical and electrophysiological characteristics i.e. waveform, firing rate and pattern (Invernizzi et al. 2007; Di Giovanni et al. 1999).

Systemic administration of two NOS inhibitors, *N*-ω-nitro-L-arginine methyl ester (L-NAME, 50 mg/kg, i.p.) and 7-nitroindazole (7-NI, 50 mg/kg, i.p.) (Fig. 1a, b), did not cause any significant change in the basal firing rate and bursting activity of the DA neurons in the SNc recorded ($n = 5$ for each drug). The effects of the drug treatment were evaluated for at least 20 min or more when it was possible, at 5-min intervals. Accordingly, treatment with the NO precursor L-arginine (L-ARG, 50 mg/kg, i.p.; $n = 5$) and the NO releaser molsidomine (MOL, 50 mg/kg, i.p.; $n = 5$) did not produce any significant modification of the neuronal discharge (Fig. 1c). Moreover, the modification of NO levels within the SNc, by L-NAME (−20 to −60 nA, pH 6.5; $n = 5$) or L-arginine (L-ARG, +40 nA for 5 min, pH 6; $n = 5$) microiontophoretic application, did not produce any modification in the discharge of SNc DA neurons (Fig. 1d). Consistent results with the extracellular recordings were obtained by neurochemical approach. In fact, 7-NI or L-NAME (50 mg/kg, i.p.; $n = 5$ each) treatment did not modify the DA release in the striatum, although DOPAC efflux was significantly reduced by the 7-NI treatment (−38.5 ± 4.8) (Fig. 2a, b).

Furthermore, neither MOL (50 mg/kg, i.p.; $n = 5$) nor L-ARG (50 mg/kg, i.p.; $n = 5$) modified DA release or DOPAC efflux in the striatum (data not shown). In contrast to this lack of effect under normal conditions, the inhibition of NO system was able to counteract the typical excitatory effect induced by treatment with nicotine on nigrostriatal function (Figs. 2 and 3). Pre-treatment with 7-NI or L-NAME

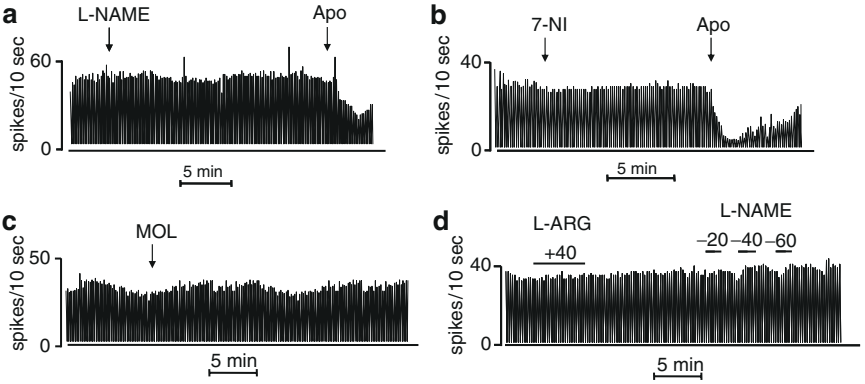

Fig. 1 Effect of systemic and local manipulation of NO signalling on the firing rate of DAergic SNc neurons. Representative rate histograms showing the effects elicited by i.p. administration (at *arrows*) of L-NAME (50 mg/kg) (**a**), 7-NI (50 mg/kg) (**b**), MOL (50 mg/kg) (**c**). APO, apomorphine administration (10 μg/kg, i.v., at *arrow*). (**d**) Typical rate histogram showing the effects elicited by locally applied ARG and L-NAME. Numbers above each bar indicate the ejecting currents in nA; the length of the bars is proportional to the time of the drug application

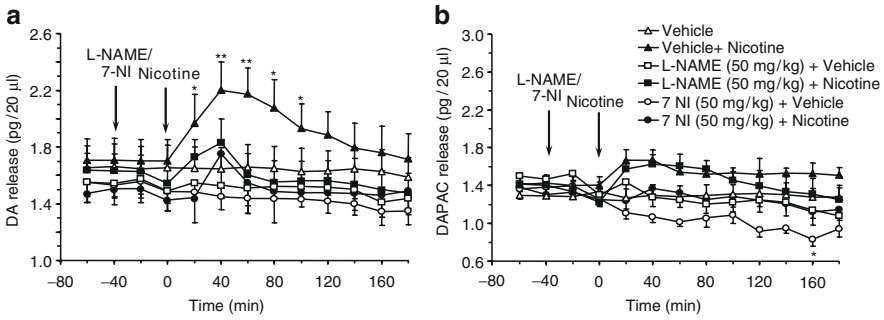

Fig. 2 Time course of the effects of 7-NI (open circle, 50 mg/kg, i.p., $n = 5$), L-NAME (open square, 50 mg/kg, i.p., $n = 5$) and nicotine (filled triangle, 1 mg/kg, i.p., $n = 5$) on extracellular DA and DOPAC levels in the striatum (**a**, **b**). Control groups treated with vehicle (inverted open triangle). The drugs were administered at the time indicated by *vertical arrows*. Each data point represents mean ± SEM absolute levels of DA, without considering probe recovery. Statistical analysis shows a significant effect of nicotine (one-way ANOVA; $p < 0.01$) as compared with the control group. 7-NI or L-NAME prevented nicotine-induced increase in DA release [two-way ANOVA; $^{*}p < 0.05$, $^{**}p < 0.01$ vehicle + nicotine as compared with 7-NI + nicotine (closed circle, $n = 5$) or L-NAME + nicotine (closed square, $n = 5$) by Tukey–Kramer's post hoc test]. In the striatum all pharmacological treatments failed to change DOPAC release, although 7-NI at 160 min reduced it significantly (one-way ANOVA; $p < 0.05$)

(50 mg/kg, i.p.) blocked the dose-dependent increase of the firing rate and the bursting activity of DA neurons in the SNc induced by acute i.v. injections of nicotine (25–400 μ/kg) (Fig. 3b–d).

As shown by the dose–response curve reported in Fig. 3d, nicotine reached its maximal effect (+93 ± 19%, above baseline) at the cumulative dose of 775 μg/kg that was statistically significant compared with the groups treated with the vehicles of 7-NI and L-NAME ($n = 5$). Pre-treatment with either 7-NI or L-NAME (50 mg/kg, i.p.), completely prevented nicotine-induced increase in DA firing rate and burst firing (the maximum effect was +34 ± 18% and +26 ± 8% after 7-NI and L-NAME treatment, respectively; Fig. 3d). In addition, the same pre-treatment with 7-NI or L-NAME (50 mg/kg, i.p.) prevented the enhancement in DA release elicited by acute nicotine (1 mg/kg, i.p.; $n = 5$) in the corpus striatum as well (Fig. 2a). In conclusion, inhibition of NOS by 7-NI or L-NAME was capable of counteracting the activation of nigral DA neurons caused by acute administration of nicotine.

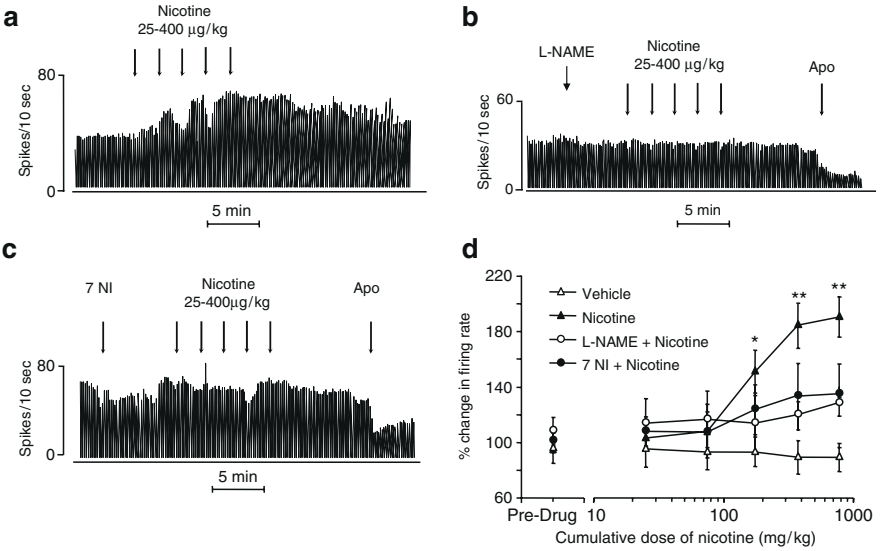

Fig. 3 Blockade by 7-NI and L-NAME of the excitatory actions of nicotine on the firing rate of SNc neurons. (**a**) Representative rate histogram showing the i.v. effect of nicotine (25, 50, 100, 200 and 400 μg/kg, at *arrows*). (**b**, **c**) Representative rate histograms showing that pre-treatment with 7-NI and L-NAME (50 mg/kg, i.p.) prevents the excitatory effect of nicotine. (**d**) Cumulative dose–response curve showing the mean percentage change (±S.E.M.) in firing rate after nicotine, vehicle, 7-NI + nicotine and L-NAME + nicotine. Statistical analysis revealed a significant effect of nicotine (one-way ANOVA; $p < 0.01$; $n = 7$) compared with the group treated with the vehicle of 7-NI and L-NAME ($n = 5$). Pre-treatment with 7-NI or L-NAME (50 mg/kg, i.p.), which did not cause any significant effect by itself (one-way ANOVA; $p < 0.29$; $n = 6$), completely prevented nicotine-induced increase in DA firing rate (two-way ANOVA; $^{*}p < 0.05$; $^{**}p < 0.01$ by Tukey–Kramer post hoc test)

5.2 Discussion

An important finding of our new work is that NO does not control DA tonic neurotransmission, in agreement with other investigations (Cox and Johnson 1998; Nowak et al. 2002; Schilström et al. 2004; Campos et al. 2006). In fact, neither the disruption of NO levels by treatment with NOS inhibitors, 7-NI and L-NAME, nor the NO elevation by L-ARG and MOL treatment was able to produce any changes in the firing rate and burst firing of SNc DA neurons. This lack of effect was obtained by general and local application of the drugs by microiontophoresis revealing that, probably, under tonic DAergic activation nigral NO does not play a relevant physiological role. Our findings are consistent with the evidence that L-NAME and L-ARG treatment did not modify the firing discharge of the ventral tegmental area (VTA) DAergic neurons in vivo (Schilström et al. 2004) and of both VTA and SNc neurons in vitro (Cox and Johnson 1998; Schilström et al. 2004). Furthermore, our neurochemical data are consistent with the electrophysiological recordings, likewise showing that endogenous nitrergic tone does not influence basal striatal DA release. The absence of effect of NO on DA function might be contingent upon confounding factors e.g. drug type-dependent effects, background redox state of the brain tissue, different NO regulation in different striatal subregions, etc. Nevertheless, we are confident in excluding limitations of the techniques since using a similar approach we have found effects of NO manipulation on the firing activity of other nuclei of the basal ganglia (Di Giovanni et al. 2003, 2006). Despite the inability of NO to modify basal DA function, the inhibition of NOS completely counteracted the stimulation of DA outflow induced by nicotine. We used nicotine because it is well known to be able to activate the nigrostriatal system at both nigral and striatal levels as well as the mesocorticolimbic system (Di Matteo et al. 2007). Consistent with previous studies, nicotine caused a robust phasic surge in nigral DAergic function due to combinatory effects. Exogenous nicotine increases the DA SNc activity in part via a direct activation of α_7-subunit containing nicotinic ACh receptors (α_7-nAChRs) present on the soma and axon of DA neurons. Simultaneously, nicotine causes a persistent depression of the inhibitory GABAergic inputs and a potentiation of glutamatergic afferents to the SNc (Di Matteo et al. 2007). The net effect is a shift toward excitation of the dopamine reward system following nicotine exposure.

It is worth highlighting that this intense nicotine DAergic activation is mediated almost completely by NO. Indeed, disrupting NO endogenous tone reduced nicotine-induced DA release by ≈60% and contextually the SNc DA neuron firing rate is similarly decreased. The mechanism by which inhibition of NOS influences the nicotine-induced activation of DA cells in the SNc observed in vivo is far from simple. It is unlikely that a direct synaptic effect on DA neurons is involved, since it has been shown that L-NAME does not alter the firing rate induced by nicotine in vitro and does not affect nicotine-induced inward currents in the VTA (Schilström et al. 2004). This assumption agrees with the anatomical evidence that only few DA neurons in the SNc express NOS machinery. Nicotine increases both firing

and burst rate of DA neurons reducing their inhibitory drive and enhancing the excitatory influence on the SNc. NO might be involved in both effects modulating GLU and GABA release in an opposite way. The phasic DA activation boosts striatal NO tone (Sammut et al. 2006, 2007) that in turn excites MSNs as well as excites SNc DA cells by disinhibiting SNr neurons and permitting the nicotine effect. At the same time, the inhibitory striatal inputs to the SNc, those that seem not to be dependent on NO, are active as well, limiting the excitatory nicotine effect. Furthermore, due to the sustained DA nicotine overflow higher levels of striatal NO eventually may result in an inhibition of the function of NMDA receptors and a decrease of the activity of MSNs projecting to the SNr/SNc (Choi and Lipton 2000; Di Giovanni et al. 2003), thus reducing the activity of indirect excitatory striatonigral pathway.

It is possible that removal of endogenous NO tone by 7-NI or L-NAME treatment might decrease the indirect excitatory pathway through the SNr balancing the direct inhibitory one, reducing GLU release and leading the SNc neurons to a hypo-functional state. Nicotine in this condition might be unable to exert its excitatory effect.

Therefore, we propose that the degree of activity of nigrostriatal DA neurons may constitute a key factor for the expression of the NO/DA interaction, in that enhanced DA synthesis and/or release would be required to permit the occurrence of a NO modulatory control. In line with this hypothesis is the evidence that striatal NO increases only when SNc neurons fire at high frequency and in burst (Sammut et al. 2006, 2007). Further, it has been shown that the permissive role played by NO in phasic DA activation is not exclusive for nicotine and is applicable to other excitatory stimuli, both in physiological (West and Grace 2000) and in pharmacological terms (Tayfun Uzbay and Oglesby 2001). Thus, NO seems to have a more general role in controlling the DA brain reward and motivation circuitries even being implicated in the placebo effect (Fricchione and Stefano 2005). Accordingly, NO system appears to be crucial in the development of dependence on different drugs of abuse such as nicotine, morphine, cocaine and alcohol. In line with this, NOS inhibitors have been shown to be able to attenuate the development and expression of the abstinence syndrome for such psychostimulants (Tayfun Uzbay and Oglesby 2001).

6 Conclusions

In summary, our neurochemical and electrophysiological results, in combination with previous findings reviewed here, further demonstrate that NO is involved in both physiological and pathophysiological processes in the nigrostriatal system. Noticeably, our evidence indicates that endogenous NO positively modulates the efflux of DA in the striatum only when DA transmission is increased above basal levels. The determination of the exact mechanisms underlying this interaction may, in the near future, open new insights for the understanding of the functional role of

the central NO system within the basal ganglia. Furthermore, these results suggest that the NO system represents an important therapeutic target for the development of agents for preventing or reducing DA neurodegeneration and in general for those conditions with an impairment of DA functions. In particular, we propose that NOS inhibitors might facilitate tobacco smoking cessation blocking the hedonic nicotine increase in DA release. Our findings are further supported by the recent evidence that bupropion, one of the effective treatments available for the cessation of smoking, seems to act inhibiting the L-arginine–NO–cGMP signaling pathway rather than having a direct effect on the DA system acting as a DAT reuptake inhibitor (Dhir and Kulkarni 2007). The challenge for pharmaceutical research now is to achieve selective inhibition of NOS isoforms, a situation complicated by the possibility that NOS inhibitors can indiscriminately affect beneficial and pathological NO signalling pathways.

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