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Research report

Signaling pathways in the nitric oxide and iron-induced dopamine release in the striatum of freely moving rats: Role of extracellular Ca^{2+} and L-type Ca^{2+} channels

Gaia Rocchitta[a], Rossana Migheli[a], Maria P. Mura[a], Giuseppe Grella[b], Giovanni Esposito[a], Bianca Marchetti[a,c], Egidio Miele[a], Maria S. Desole[a], Maddalena Miele[d], Pier Andrea Serra[a,*]

[a]Department of Pharmacology, University of Sassari, viale S.Pietro 43B, 07100 Sassari, Italy
[b]Department of Pharmaco-chemical Toxicology, University of Sassari, via Muroni 29, 07100 Sassari, Italy
[c]OASI Institute for Research and Care on Mental Retardation and Brain Aging (IRCCS), Neuropharmacology Section, 94018 Troina, Italy
[d]Department of Psychiatry, St. Mary's Hospital, London, UK

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Abstract

We showed previously that exogenous iron potentiated nitric oxide (NO) donor-induced release of striatal dopamine (DA) in freely moving rats, using microdialysis. In this study, the increase in dialysate DA induced by intrastriatal infusion of the NO-donor 3-morpholinosydnonimine (SIN-1, 1.0 mM for 180 min) was scarcely affected by Ca^{2+} omission. *N*-methyl-D-glucamine dithiocarbamate (MGD) is a thiol compound whose NO trapping activity is potentiated by iron(II). Intrastriatal co-infusion of MGD either alone or associated with iron(II), however, potentiated SIN-1-induced increases in dialysate DA. In contrast, co-infusion of the NO trapper 4-(carboxyphenyl)-4,4,5,5-tetramethylimidazole-1-oxyl 3-oxide (carboxy-PTIO) significantly attenuated the increase in dialysate DA induced by SIN-1 (5.0 mM for 180 min). SIN-1+MGD+iron(II)-induced increases in dialysate DA were inhibited by Ca^{2+} omission or co-infusion of either deferoxamine or the L-type (Ca_v 1.1–1.3) Ca^{2+} channel inhibitor nifedipine; in contrast, the increase was scarcely affected by co-infusion of the N-type (Ca_v 2.2) Ca^{2+} channel inhibitor ω-conotoxin GVIA. These results demonstrate that exogenous NO-induced release of striatal DA is independent on extracellular Ca^{2+}; however, in presence of the NO trapper MGD, NO may preferentially react with either endogenous or exogenous iron to form a complex which releases striatal DA with an extracellular Ca^{2+}-dependent and nifedipine-sensitive mechanism.

Theme: Calcium channel physiology, pharmacology and modulation
Topic: Mechanism of neurotransmission release

Keywords: Exogenous nitric oxide; Iron; Complex; Calcium channels; Striatal dopamine release; Parkinson's disease

1. Introduction

Nitric oxide (NO) is a versatile and widespread biological messenger molecule. NO signaling plays an important role in the functioning of the central nervous system (CNS) [11]. Activation of soluble guanylate cyclase (sGC) is one of the main intracellular effects of NO. The resulting increase in cyclic GMP modulates, among other activities, that of ion channels [20]. The modulation of ion channels, including Ca^{2+} channels and pores, is emerging as a general mechanism by which NO exerts biological signaling [18]. Several types of Ca^{2+} channels coexist to regulate neurotransmitter release in the CNS [54]. Physiological dopamine (DA) release from dopaminergic terminals has been shown to be largely dependent on N-type (Ca_v 2.2) and P/Q-type (Ca_v 2.1) voltage-dependent Ca^{2+} channels [5,35]. A variety of in vivo studies, in which NO-generating drugs were preferentially employed, have shown that NO modulates extracellular levels of DA in the rat striatum [12,44,46–

* Corresponding author. Fax: +39 79 228525.
E-mail address: pharmaco@uniss.it (P.A. Serra).

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48,58]. In a previous study, we demonstrated that exogenous NO-induced striatal DA release from the rat striatum in vivo was independent of both sGC/cyclic GMP pathway activation and extracellular Ca^{2+} [44].

Understanding the chemistry of NO is important in order to clarify the activity of NO in the striatal DA release in vivo. NO is a simple hydrophobic gaseous molecule that is highly diffusible and reactive. The following forms are important for its biological action: NO· radical, which can be oxidized to nitrosonium cation (NO^+), or reduced to nitronyl anion (NO^-) [53]. NO readily reacts with either iron(II) to form Fe(III)–NO^- complexes, or with iron(III) to form Fe(II)–NO^+ complexes [23,53]. Iron is a transitional metal involved in many catalytic and regulatory neuronal processes [60], but unless appropriately shielded, it can promote oxidative stress through reactive oxygen species formation [16]. NO may behave either as pro-oxidant [2] or antioxidant [17] in the iron-mediated oxidative stress. In turn, iron may protect tumor cell from pro-apoptotic effects of NO [9]. Interestingly, either NO-donors, with the exception of sodium nitroprusside (SNP), or NO gas in Ringer's solution, protected nigral neurones from iron(II)-induced oxidative stress [25,29,37].

The composition of the endogenous environment in which NO is generated [28,39,50,51], as well as the timing of NO generation [59] may also regulate its biological actions. In this regard, ascorbic acid is a very important component of the endogenous environment. Neuronal ascorbic acid concentrations (10 mM) are about 10 times higher than glial and, respectively, 20–25 times higher than extracellular concentrations [27,40]. The close relationship between NO and ascorbic acid has been outlined in several biological systems [24,28]. NO is readily oxidized to nitrite (NO_2). Ascorbic acid may reduce nitrite ions (NO_2^-) back to NO in the extracellular space [28]. In addition, ascorbic acid protects NO from destruction by superoxide anion ($O_2^{\bullet -}$) [8,15].

We showed previously that interaction between NO and iron(II), both released following the decomposition of SNP, accounted for the late SNP-induced DA increase in dialysates from the striatum of freely moving rats [46]; in addition, we showed that co-infusion of iron(II) with either the NO-donor S-nitroso-N-acetylpenicillamine [47] or the NO-donor and potential peroxynitrite generator 3-morpholinosydnonimine (SIN-1) [48] mimicked SNP effects on striatal DA release. Adding information on the mechanism of the iron/NO complex-induced release of striatal DA has been the aim of the present research.

2. Materials and methods

2.1. Sources of compounds

Nifedipine (NIF), 4-(Carboxyphenyl)-4,4,5,5-tetramethylimidazole-1-oxyl 3-oxide (carboxy-PTIO), 3-morpholinosydnonimine (SIN-1), S-nitroso-N-acetylpenicillamine (SNAP), deferoxamine, and ferrous sulphate [$FeSO_4$, iron(II)] were purchased from Sigma-Aldrich (Milano, Italy); *N*-methyl-D-glucamine dithiocarbamate (MGD) was synthesized by one of us (G. G.); ω-conotoxin GVIA (ω-C-GVIA) from was purchased from Tocris Cookson (Bristol Avon, UK).

2.2. Animals

Male Wistar rats (Morini, R. Emilia, Italy), weighing between 280 and 330 g were used in all experiments. The rats were maintained under standard animal care conditions (12:12 h light/dark cycle, lights coming on at 7 a.m.; room temperature 21 °C), with food and water ad libitum. Prior to the start of any experiment, the health of the rat was assessed according to published guidelines [31]. All procedures were specifically licensed under the European Community directive 86/609 included in Decreto No. 116/1992 of the Italian Ministry of Public Health.

2.3. Microdialysis probe construction

The striatal probe (Fig. 1), which combines two independent microdialysis probes of concentric design with two separate inlets and two separate outlets, has been previously described in detail [43,44]. The two inlets with two corresponding separate outlets permit separate co-infusion of drugs and separate dialysate sample collection from the same intrastriatal site. Separate sample collection is useful when one or more drugs which may have either pro-

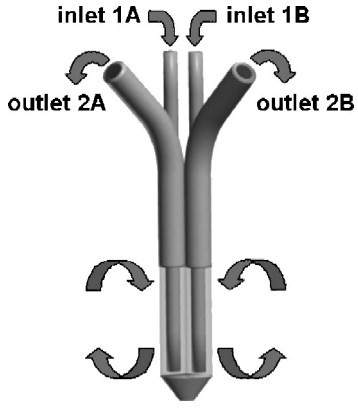

Fig. 1. Drawing of a striatal probe combining two independent microdialysis probes of concentric design with two separate inlets and two corresponding outlets. Each probe has a final diameter of 0.22 mm. The semipermeable membrane has an active length of 4.0 mm. The diameter of the final probe is approximately 0.45 mm. The main drug is infused through the inlet 1A, conventionally indicated as ipsilateral inlet, while another drug may be co-infused through the inlet 1B, conventionally indicated as contralateral inlet. Both drugs diffuse through the 4 mm of the pertinent dialytic membrane and reach the extracellular compartment. Thereafter, the concentration of neurochemicals is determined separately in dialysates collected from both outlets (2A, conventionally indicated as ipsilateral outlet, and 2B, conventionally indicated as contralateral outlet).