

Inhibition of glutamate reuptake potentiates endogenous nitric oxide-facilitated dopamine efflux in the rat striatum: an in vivo microdialysis study

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Abstract

The current study investigated the effects of the nitric oxide synthase (NOS) substrate, N^G-hydroxy-L-arginine (H-ARG) and the selective glutamate (GLU) reuptake inhibitor (2S)-trans-pyrrolidine-2,4-dicarboxylic acid (PDC) on striatal dopamine (DA) and glutamate (GLU) efflux in vivo. Concentric microdialysis probes were stereotaxically implanted in the anterior-medial striatum of chloral hydrate-anesthetized rats. Intra-striatal infusion of PDC (200 μM) elevated extracellular (EC) DA and GLU levels concurrently over a 10 fraction collecting period without affecting EC asparagine levels. Infusion of H-ARG (200 μM) for six 20-min fractions, also significantly elevated EC DA levels. In the presence of PDC (200 μM), co-perfusion of H-ARG (200 μM) resulted in supra-additive increases in EC DA levels. The synergistic effect of PDC and H-ARG infusion on DA efflux was attenuated by co-infusion of the NOS inhibitor, 7-nitroindazole (100–200 μM). These results suggest that while both endogenous NO and GLU regulate striatal DA efflux via facilitatory influences, enhanced glutamatergic tone on striatal NOS-containing neurons may potentiate NO-synthesis and subsequently NO-induced DA efflux. © 1997 Elsevier Science Ireland Ltd.

Keywords: Dopamine; Glutamate; Nitric oxide; Nitric oxide synthase; Microdialysis; Striatum; N^G-hydroxy-L-arginine; (2S)-trans-Pyrrolidine-2,4-dicarboxylic acid

Nitric oxide is a freely diffusible, non-polar, radical, gas produced enzymatically in multiple cell types in the central nervous system. Several distinct isoforms of the NO producing enzyme, nitric oxide synthase (NOS), have recently been described (see [17] for review). Of these isoforms, type 1 (neuronal) possesses constitutive activity and has been localized within the rat striatum [2].

Recently, striatal NOS interneurons have been shown to receive afferent inputs from dopaminergic nigrostriatal terminals and corticostriatal inputs [4,18]. As these NO producing cells are evenly distributed throughout the striatum and have a dense plexus of axon collaterals, they are in position to modulate the activity of multiple striatal cell types and afferent inputs [18]. Given the multiple reports that GLU and other EAAs can increase NOS activity via *N*-

methyl-D-aspartate (NMDA) receptor-activated calcium influx (see [5] for review), these NO-producing cells may mediate some of the actions of the excitatory corticostriatal afferent pathway involved in controlling striatal function. In support of this, activation of cortical afferents enhances dye coupling in rat striatal neurons via a NO-mediated modulation of gap junction permeability [11].

Although the neuroanatomical and immunocytochemical characteristics of striatal NOS containing cells have been well studied, few investigations exist describing the functional interaction of these cells with intrinsic and afferent striatal neurotransmitter systems in vivo. We have recently demonstrated that infusion of the immediate NO precursor N^G-hydroxy-L-arginine (H-ARG) [1,16], facilitates striatal dopamine (DA) and glutamate (GLU) efflux in a manner dependent on neuronal NOS activity [20]. In the current study, we have investigated the influence of striatal NO production and modulation of corticostriatal glutamatergic inputs on extracellular (EC) GLU, asparagine (ASN), and DA levels via infusion of H-ARG and the selective GLU

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reuptake inhibitor (2S)-trans-pyrrolidine-2,4-dicarboxylic acid (PDC).

In these studies adult, male Harlan Sprague–Dawley rats (Indianapolis, IN) were housed under conditions of constant temperature (21–23°C) and maintained on a 12 h light/dark cycle with food and water provided ad libitum. All animal procedures were approved by the Wayne State University Animal Investigational Committee and adhere to the NIH Guide for the care and use of laboratory animals. Microdialysis and high pressure liquid chromatography with electrochemical detection (HPLC-ED) of DA were performed as described previously [19,20]. Microdialysis probes (4 × 0.15 mm of exposed membrane) of concentric design were stereotaxically placed in the anterior-medial striatum of chloral hydrate-anesthetized male rats. The method of Donzanti and Yamamoto [3] was used to determine striatal dialysate amino acid levels. Briefly, the dialysate amino acid levels were determined via HPLC-ED following a pre-column ortho-phthalaldehyde (OPA)-β-mercaptoethanol (βME) derivatization procedure. The stock derivatizing reagent contained 27 mg OPA, 1 ml methanol, 5 μl βME, 9 ml 0.1 M sodium tetraborate (pH = 9.4). This reagent was diluted each day with 0.1 M sodium tetraborate (1:3) and then added to standards and dialysate samples (1:2) approximately 2 min prior to analysis by HPLC-ED. H-ARG, methanol, OPA, sodium tetraborate, and βME were purchased from Sigma Chemical Co. (St. Louis, MO). (2S)-trans-pyrrolidine-2,4-dicarboxylic acid was purchased from Research Biochemicals Inc. (Natick, MA). 7-Nitroindazole monosodium (7-NI) was purchased from CalBiochem (La Jolla, CA). All drugs were readily dissolved in artificial cerebral spinal fluid (aCSF).

Baseline GLU, ASN, and DA levels were determined and the average of three stable fractions immediately preceding drug treatment and not varying by 20% in analyte concentration (fmol/μl), was set as the control (100%) unless indicated otherwise. Data are expressed as percent of control and values are not adjusted for probe recovery. The statistical significance of potential PDC and H-ARG-induced changes in basal EC DA, GLU, and ASN concentrations was assessed using either a one-way repeated measures analysis of variance (ANOVA-RM) or ANOVA with post-hoc Dunnett's multiple comparisons test as indicated.

Fig. 1 depicts the time course and effects of intra-striatal PDC (200 μM) infusion on EC GLU and ASN concentrations. In these experiments, following baseline determination PDC infusion via the probe for ten 20-min fractions resulted in an immediate 186% increase in EC GLU levels (**P* < 0.05, *n* = 4–6) without affecting EC ASN levels (*P* > 0.05, *n* = 5–6). The PDC-induced increase in EC GLU levels stabilized over the ten fraction delivery period at approximately 200% above basal levels (**P* < 0.05, *n* = 4–6). Following removal of PDC from the perfusate, EC GLU levels returned to basal levels.

As shown in Fig. 2, following the determination of basal DA levels, intra-striatal PDC (200 μM) infusion increased

EC DA levels to approximately 200–250% of basal levels over an 80-min period (**P* < 0.05, *n* = 6). After PDC (200 μM) pretreatment, infusion of H-ARG and PDC elevated EC DA levels to approximately 500% of basal levels during the next six fractions (**P* < 0.05, *n* = 6). In control experiments, PDC (200 μM) infused for ten 20-min fractions, increased EC DA levels to approximately 250% of basal levels (**P* < 0.05). Following removal of drug from the perfusate, DA levels returned towards basal levels over a 60-min recovery period. As shown in the inset to Fig. 2, H-ARG (200 μM) infused for six 20-min fractions increased EC DA levels 79% over basal levels (**P* < 0.05, *n* = 10). When H-ARG and PDC (200 μM) were co-perfused following PDC pretreatment, striatal EC DA levels increased to approximately 500% of basal levels, a significant potentiation over the effects observed with either drug alone (***P* < 0.05).

In order to determine whether PDC/H-ARG-mediated DA efflux is occurring via a NOS-dependent mechanism, the NOS inhibitor 7-NI (100–200 μM) was co-infused with PDC over an 80-min period and co-perfused along with PDC and H-ARG for an additional six 20-min fractions. As demonstrated in Fig. 3 (top), the addition of 7-NI (100–200 μM) to the perfusate did not influence PDC-mediated DA efflux (*P* > 0.05, *n* = 4–5). However, as shown in Fig. 3 (bottom), co-perfusion with the 100 and 200 μM concentrations of 7-NI significantly attenuated the supra-additive enhancement of DA efflux observed during H-ARG and PDC co-infusion (**P* < 0.05, *n* = 4–5).

In the current study, intra-striatal PDC infusion elevated EC GLU levels without affecting EC ASN concentrations, demonstrating the specificity of PDC as a selective GLU transporter inhibitor. These results are in agreement with a

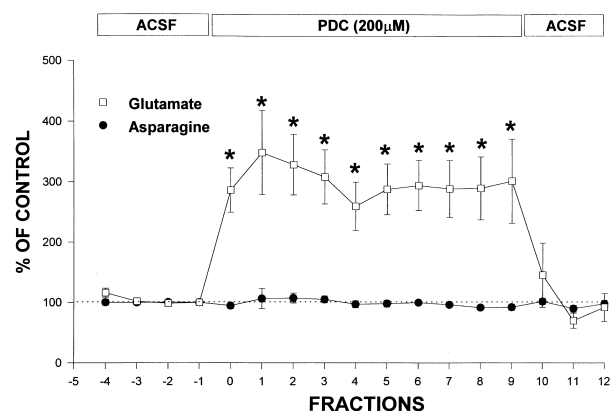


Fig. 1. Time course of PDC and PDC + H-ARG-induced striatal GLU release. Intra-striatal PDC (200 μM) infusion increased EC GLU levels maximally to $348 \pm 69\%$ of basal levels during a ten fraction delivery period (**P* < 0.05). In contrast, intra-striatal PDC-administration did not significantly affect EC ASN levels (*P* > 0.05). Symbols represent the mean \pm SEM from *n* = 4–6 experiments. Asterisks indicate values that differ significantly from baseline GLU levels as determined by ANOVA-RM with a Dunnett's post-hoc test. Mean \pm SEM basal GLU and ASN levels for these groups were 128 ± 18 and 186 ± 19 fmol/μl, respectively.

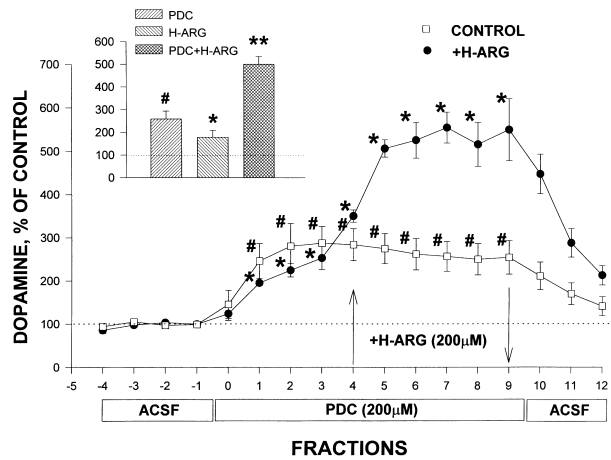


Fig. 2. Time course of PDC and PDC + H-ARG-induced striatal DA release. Intra-striatal PDC (200 μM) infusion maximally increased EC DA levels by $153 \pm 27\%$ over an 80-min period. H-ARG (200 μM) co-perfused with PDC (200 μM) maximally elevated EC DA levels to $555 \pm 36\%$ of basal levels ($*P < 0.05$). PDC (200 μM) infused alone for ten 20-min fractions increased EC DA levels maximally during the third fraction to $288 \pm 38\%$ of basal levels ($*P < 0.05$). EC DA levels remained elevated during the entire ten fraction PDC-administration period ($*P < 0.05$). Symbols represent the mean \pm SEM from $n = 6$ experiments. Asterisks and pound signs indicate values that differ significantly from baseline DA levels as determined by ANOVA-RM with a Dunnett's post-hoc test. Inset: Supra-additive increase in EC DA following H-ARG and PDC co-perfusion. Intra-striatal infusion of H-ARG (200 μM) for six 20-min fractions increased EC DA levels to $179 \pm 30\%$ ($*P < 0.05$, determined using ANOVA with Dunnett's post-hoc test). During the final six fractions of PDC (200 μM) infusion (derived from PDC control time course), EC DA levels increased to approximately $259 \pm 35\%$ of basal levels ($*P < 0.05$, determined using ANOVA with Dunnett's post-hoc test). Following PDC (200 μM) pretreatment, H-ARG and PDC co-perfusion potentiated DA release over that of separate H-ARG and PDC infusions to $501 \pm 75\%$ of basal levels ($**P < 0.05$, determined using ANOVA with Dunnett's post-hoc test). Bars represent the mean \pm SEM DA levels in percent of control for the final six 20-min fractions during the indicated drug delivery period ($n = 6-10$ experiments). Mean \pm SEM basal DA levels for the PDC, H-ARG, and PDC + H-ARG experiments were 2.67 ± 0.17 , 1.94 ± 0.27 , and 2.58 ± 0.24 fmol/ μl , respectively.

recent in vivo microdialysis study by Massieu et al. [7], which demonstrated a dose-dependent increase in EC GLU and aspartate levels but little or no effects on EC alanine or glycine levels following intra-striatal PDC (1–25 mM) administration. Additionally, using biochemical and histological methods these investigators also demonstrated that inhibition of GLU uptake systems, using doses of PDC (and other GLU uptake blockers) 5–50 times greater than those used in the current study, did not induce neuronal damage [7].

If under the current protocol, PDC infusion was influencing striatal EC GLU levels via a neurotoxicity-mediated release process, it might be expected that the loss of cell membrane potential would result in the efflux of cellular amino acids and proteins into the interstitial space. However, as PDC infusion did not alter striatal EC ASN levels, a neurotoxic release process is not supported. Also, our results demonstrating that PDC-induced GLU efflux stabilizes over the 10 fraction drug delivery period and that EC GLU levels

return to basal concentrations following removal of drug from the perfusate, suggest intra-striatal PDC infusion is not inducing a neurotoxic insult to striatal tissues.

Previous studies have shown that enhanced striatal glutamatergic (see [6] for review) or nitrergic tone [10,14,15,19,20] can facilitate DA release in vivo. The present study is the first demonstrating that enhancement of endogenous glutamatergic tone via inhibition of GLU reuptake can facilitate striatal DA efflux separately and in a synergistic manner during NOS substrate (H-ARG) co-infusion. Recently, it has been demonstrated that H-ARG is converted to NO via an NOS-dependent reaction [1] and the type 1 NOS inhibitor 7-NI attenuates H-ARG-mediated DA release [20]. Given the above, it is likely that the elevations in EC DA levels observed following intra-striatal H-ARG

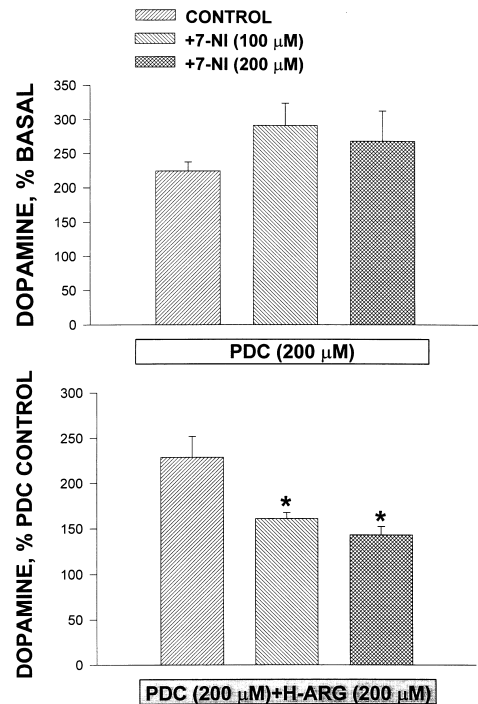


Fig. 3. The effects of 7-NI co-perfusion on PDC and PDC + H-ARG-induced striatal DA release. Top: Intra-striatal PDC (200 μM) infusion (80 min) increased EC DA levels to $224 \pm 13\%$ of basal EC DA levels (control). Co-perfusion of PDC (200 μM) with 7-NI (100 or 200 μM) increased EC DA levels to $291 \pm 33\%$ and $268 \pm 44\%$ of basal levels, respectively ($P > 0.05$, as determined using ANOVA). Bars represent the mean \pm SEM DA levels in percent of basal for the final three 20-min fractions of the PDC control or PDC + 7-NI drug delivery period ($n = 4-6$ experiments). Bottom: In control studies, H-ARG (200 μM) co-perfused with PDC (200 μM) elevated EC DA levels to $229 \pm 23\%$ of PDC pretreatment control levels. Co-perfusion of 7-NI (100 and 200 μM) attenuated the H-ARG/PDC-mediated enhancement of EC DA levels to $161 \pm 7\%$ and $143 \pm 9\%$ of PDC/7-NI pretreatment control levels ($*P < 0.05$, as determined using ANOVA with Dunnett's post-hoc test). Bars represent the mean \pm SEM DA levels in percent of PDC/7-NI pretreatment control levels for six 20-min fractions during the indicated drug delivery period ($n = 4-6$ experiments). Mean \pm SEM basal DA levels for the control, 7-NI (100 μM) + PDC + H-ARG, and 7-NI (200 μM) + PDC + H-ARG experiments were 2.58 ± 0.24 , 1.96 ± 0.18 , and 2.11 ± 0.43 fmol/ μl , respectively.

and H-ARG + PDC infusion result in part from an enhanced facilitatory nitric tone on dopaminergic neurotransmission. This is supported by the current results demonstrating that 7-NI attenuates the supra-additive stimulatory effects of H-ARG and PDC co-infusion on striatal DA efflux.

In addition to the facilitatory influence of NO on dopaminergic neurotransmission, a secondary NO-mediated enhancement of GLU efflux may play a role in the observed supra-additive effects of PDC and H-ARG co-administration on DA efflux. We have previously shown that both endogenous and exogenous NO can increase striatal DA release via a GLU receptor-dependent mechanism [19,20]. When considered along with the current results demonstrating that PDC-mediated increases in EC GLU facilitate DA efflux, these studies suggest that a glutamatergic pathway may be partly responsible for the synergistic effects of PDC and H-ARG co-perfusion on DA efflux. As 7-NI co-perfusion did not affect PDC-facilitated DA release, this effect is probably mediated via a non-nitric glutamatergic pathway. Further experiments using GLU receptor subtype selective antagonists are necessary to determine the precise pathway(s) involved in PDC-facilitated DA release.

The potentiation of DA efflux observed following co-perfusion of PDC and H-ARG may be partially explained by the development of a positive feedback cycle where, in the presence of NOS substrate, increased striatal glutamatergic tone bolsters calcium-dependent NOS activity. The resulting increase in NO output and subsequently, NO-facilitated GLU release may lead to further increases in striatal NOS activity, perpetuating the cycle. This is supported by studies showing that NO enhances striatal EC GLU levels [20], possibly via inhibition of the GLU transporter [12]. Also, in hippocampal synaptosomes NO can facilitate the docking and fusion of GLU containing vesicles to the plasma membrane [8,9,13], promoting GLU release. Thus, the coupling of an enhanced facilitatory glutamatergic tone on striatal NOS activity to NOS substrate infusion may result in increased NO-mediated DA efflux via both GLU and NO-dependent mechanisms. Further studies examining the role of GLU in the potentiation of tonic DA release during H-ARG and PDC co-perfusion are currently underway in our laboratory.

In summary, the current study demonstrates that intrastriatal infusion of the selective GLU reuptake inhibitor, PDC, enhances GLU and DA efflux *in vivo*. Additionally, co-perfusion of the NOS substrate, H-ARG, with PDC markedly elevates EC DA levels beyond that observed with either agent alone and in a manner sensitive to NOS inhibition. These results suggest a complex interaction exists between striatal nitric, glutamatergic, and dopaminergic systems.

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