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Nitric oxide increases dopamine and serotonin release in the medial preoptic area

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NITRIC oxide (NO) is becoming recognized as an important intercellular messenger in the brain. The present experiment used microdialysis to examine the potential role of NO in the regulation of dopamine (DA) and serotonin (5-HT) release in the medial preoptic area (MPOA) of freely moving male rats. The NO precursor L-arginine (L-Arg, 100 μM), administered into the MPOA via the dialysis probe, increased extracellular levels of DA, 5-HT, and the major metabolites of DA. These increases were blocked by the coadministration of the NO synthase inhibitor *N*-monomethyl L-arginine (NMMA, 400 μM). The inactive isomer D-arginine (100 μM) was ineffective, and NMMA by itself decreased DA below baseline levels. Thus, NO may modulate the release of DA and 5-HT in the MPOA.

Key words: Nitric oxide; Dopamine; Serotonin; Medial preoptic area; Microdialysis; L-arginine; *N*-monomethyl L-arginine

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Introduction

Nitric oxide (NO), originally recognized as a vasodilator released from vascular endothelial cells,[1,2] was subsequently discovered to be a neuronal messenger.[3] NO is a highly reactive gas, produced by the action of NO synthase on L-arginine (L-Arg) in a Ca^{2+} dependent fashion. In the brain NO has been implicated in long term potentiation,[4] long term depression,[5] and the development of functional cortical maps.[6] NO has also been reported to increase the release of several neurotransmitters, including dopamine (DA) from striatal slices,[7–9] norepinephrine (NE) and acetylcholine (ACh) from hippocampal slices,[10] glutamate and aspartate from the medulla,[11] and ACh from basal forebrain.[12]

The medial preoptic area (MPOA) is important for the regulation of temperature, male sexual behavior, and maternal behavior.[13–15] Large fibers containing NO synthase are scattered through the MPOA.[16] Furthermore, endogenously formed NO may mediate the hyperthermic effects of prostaglandin in the MPOA.[17]

The present study evaluated the potential role of NO in the regulation of DA and serotonin (5-HT) release in the MPOA of conscious, freely moving rats. The precursor of NO, L-arginine (L-Arg), its inactive isomer, D-arginine (D-Arg), and/or an inhibitor of its synthesis, *N*-monomethyl L-arginine (NMMA), were perfused into the brain through a microdialysis membrane, while DA, 5-HT, and their metabolites diffused into the dialysate and were assayed using high performance liquid chromatography with electrochemical detection (HPLC-EC).

Materials and Methods

Twenty-three adult male Long–Evans rats (Harlan Sprague–Dawley/Blue Spruce), weighing 300–375 g, were housed individually in large plastic cages. A 14:10 light:dark cycle was in effect, with lights out at 11.00 h. Food and water were available *ad libitum*. The animals were anesthetized with ketamine hydrochloride (50 mg kg^{-1}) and xylazine hydrochloride (4 mg kg^{-1}). Each animal received a 21 ga stainless steel guide cannula, ending above the left MPOA (AP, +2.4; ML, +0.2; DV, −7.0; incisor bar, +5 mm). Details of the surgery and cannula construction are described in reference 18. A 250 μl centrifuge tube with the end cut off (approximate length 8 mm) was imbedded in the dental cement around the guide cannula to provide a seat for the microdialysis probe. An obturator fashioned from 26 ga stainless steel tubing was inserted though a centrifuge tube cap and was cut to end even with the guide cannula.

Microdialysis probes using a concentric flow design were made according to the procedure in reference 19. A length of silicon capillary tubing (150 μm o.d.) was inserted into a 17 mm 26 ga stainless steel tube extending approximately 1 mm beyond the tip of the stainless steel tube. A hollow fiber dialysis membrane (MW cutoff, 6000; 210 μm o.d.; Spectra-Por) was fitted over the silicon capillary tube and glued to the tip of the stainless steel tube. The length of the dialysis membrane was cut to 1.5 mm and plugged with waterproof epoxy. The active dialyzing surface of the membrane was 1 mm. Perfusion medium flowed in through the stainless steel tubing, and out via the silicon capillary tube, surrounded by medline tubing, into a 250 μl centrifuge tube. *In vitro* recovery averaged 30% at the rate of flow used in these experiments.

The dialysis perfusion medium was a modified Dulbecco's phosphate buffered saline solution (Sigma) (138 mM NaCl, 2.7 mM KCl, 0.5 mM $MgCl_2$, 1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, and 1.2 mM $CaCl_2$; pH 6.5). Perfusion flow was controled by a Harvard

syringe infusion pump (model 22) at a rate of 0.3 μl min^{-1}. Dialysates were collected every 20 min. On the day of testing, the animal was briefly anesthetized with ketamine hydrochloride (25 mg kg^{-1}) and xylazine hydrochloride (2 mg kg^{-1}) to allow removal of the obturator and insertion of the probe. The flow of perfusion medium was started immediately after probe insertion, and a 2 h stabilization period occurred prior to sample collection. Baseline samples were collected until three consecutive samples showed no more than 10% variation in levels. At this time the injection syringe was exchanged for one containing the appropriate drug dissolved in Dulbecco's solution, and three samples were collected during drug infusion. Sixteen animals received the NO precursor L-Arg (100 μM); half of these also received the inactive isomer D-Arg (100 μM) for three samples before L-Arg administration. The remaining seven animals received the NO synthesis inhibitor NMMA (400 μM); four of these also received NMMA plus L-Arg (100 μM) following NMMA alone. At the conclusion of the experiment, subjects were sacrificed, the brains were frozen, and the probe placements were histologically verified.

Samples were assayed for the parent amines DA and 5-HT and their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) using HPLC-EC. Compounds were separated in a BAS Sep-Stik microbore column, using a mobile phase consisting of 32 mM citric acid, 54.3 mM sodium acetate, 0.074 mM Na_2EDTA, 0.215 mM octyl sodium sulfate and 3% methanol (pH, 4.1). A Gilson model 307 pump, equipped with a flow splitter and three pulse dampers, operated at a flow rate of 0.62 ml min^{-1}. Compounds were detected with a BAS LC-4B amperometric detector (sensitivity, 0.5 nA/V), using a glassy carbon electrode maintained at a potential of +0.7 V relative to a Ag/AgCl reference electrode. The limit of detectability (3 × background) was 0.5 ng 5 μl^{-1}.

The data are expressed as a percentage of the final baseline sample preceding the drug treatment. Separate analyses of variance (ANOVAs) were run for the two groups of animals, followed by Duncan's *post hoc* comparisons. The first ANOVA compared baseline, the first D-Arg, and the first L-Arg samples; the second compared baseline, the first NMMA, and the first NMMA+L-Arg samples. All animals had probes correctly placed in the MPOA.

Results

L-Arg significantly increased extracellular levels of DA [$F(2,37) = 6.36$, $p < 0.005$], its metabolites DOPAC [$F(2,37) = 6.89$, $p < 0.005$], and HVA [$F(2,37) = 3.85$, $p < 0.05$], and also 5-HT [$F(2,37) = 3.30$, $p < 0.05$] (see Fig. 1). A slight increase in 5-HIAA levels during L-arginine administration was not statistically significant. All of these increases were completely blocked by coadministration of the NO synthase inhibitor, NMMA. The inactive isomer D-Arg was ineffective. NMMA decreased DA below baseline levels [$F(2,15) = 7.35$, $p < 0.01$; see Fig. 1], sug-

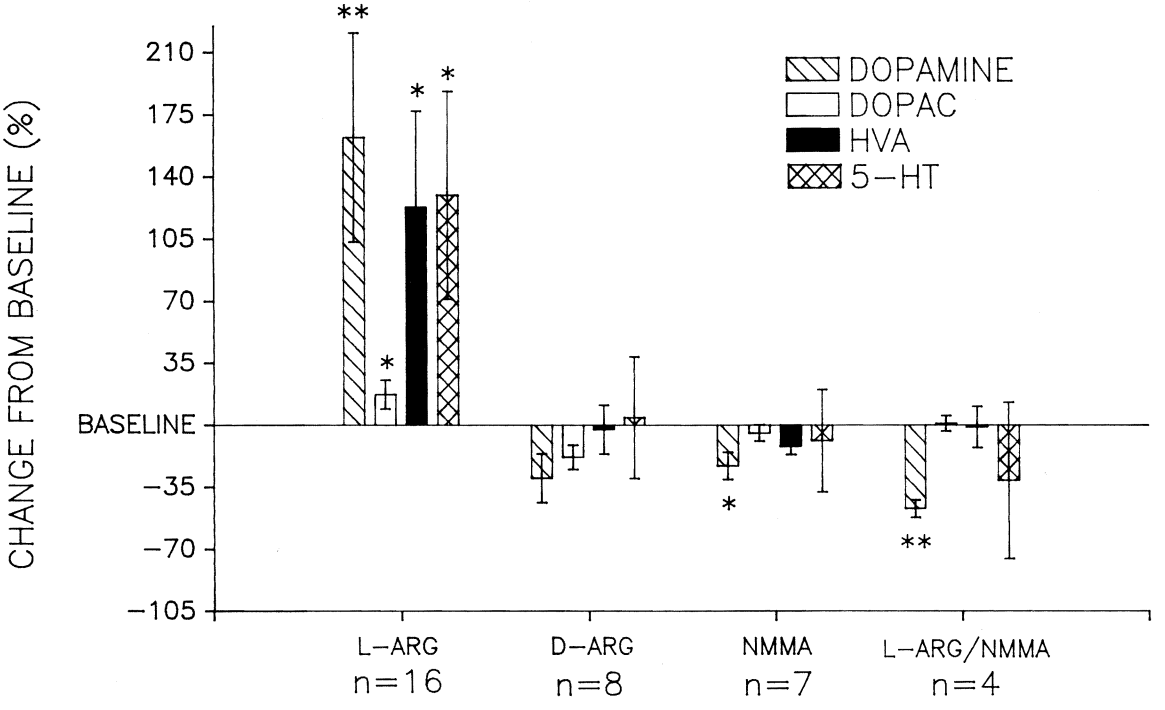

FIG. 1. The effects of L-arginine, D-arginine, and *N*-monomethyl L-arginine on extracellular levels of dopamine, its major metabolites, and serotonin in the medial preoptic area. Values are means (± standard errors) of the percentage of the final baseline level. $^{**}p < 0.01$ $^{*}p < 0.05$.

gesting that endogenous levels of NO may promote DA release.

Discussion

L-Arg, the precursor of NO, stereospecifically increased the release of DA and its major metabolites and also enhanced the release of 5-HT. In addition, the NO synthase inhibitor NMMA inhibited the basal release of DA and abolished the facilitation by L-Arg. These data suggest that endogenously formed NO may facilitate the release of these monoamines. A likely reason that NMMA+L-Arg produced slightly more inhibition than did NMMA alone is that the combination was always given after NMMA alone. Thus, there was a progressive inhibition of NO synthesis over time. Furthermore, the synthesis inhibitor was four times as concentrated as the precursor, so that L-Arg would have little chance to increase NO synthesis.

The means by which NO influences transmitter release is not clear. Because NO synthase is located in scattered large fibers in the MPOA,[16] it may elicit the release of transmitter(s) from those same fibers, or, since NO readily permeates cell membranes, it may affect release from nearby terminals. Activation of guanylate cyclase appears to mediate NO's enhancement of excitatory amino acid release in the medulla;[11] however, neither activation of guanylate cyclase nor blockade of electron transport was implicated in its induction of Ca^{2+}-dependent DA release from striatal slices.[8]

DA activity in the MPOA is increased during copulation in male rats.[20,21] Furthermore, stimulation of DA receptors in the MPOA facilitates copulation,[18] ex copula genital reflexes,[22,23] and sexual motivation.[24] In addition, 5-HT levels are increased in tissue punches of male rats following ejaculation, and may contribute to the postejaculatory period of quiescence.[25] Thus, one function of NO in the MPOA may be to modulate the prolonged DA release that accompanies and facilitates copulation and to elicit the release of 5-HT at ejaculation.

Conclusion

Administration of L-Arg, the precursor of NO, into the MPOA of male rats increased extracellular levels of DA and its major metabolites, as well as 5-HT. The inactive isomer was ineffective. The NO synthase inhibitor NMMA decreased basal levels of extracellular DA and blocked the ability of L-Arg to increase release. Thus, endogenously formed NO may enhance the release of neurotransmitters in the MPOA, which have been implicated in the control of male sexual behavior and of temperature regulation.

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