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NITRIC OXIDE MEDIATES GLUTAMATE-EVOKED DOPAMINE RELEASE IN THE MEDIAL PREOPTIC AREA

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Abstract—Dopamine (DA) release in the medial preoptic area (MPOA) of the hypothalamus is an important facilitator of male sexual behavior. The presence of a receptive female increases extracellular DA in the MPOA, which increases further during copulation. However, the neurochemical events that mediate the increase of DA in the MPOA are not fully understood. Here we report that glutamate, reverse-dialyzed into the MPOA, increased extracellular DA, which returned to baseline after the glutamate was removed. This increase was prevented by co-administration of the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME), but not by the inactive isomer, N*w*-nitro-D-arginine methyl ester (D-NAME). In contrast, extracellular concentrations of the major metabolites of DA were decreased by glutamate, suggesting that the DA transporter was inhibited. These decreases were also inhibited by L-NAME, but not D-NAME.

These results indicate that glutamate enhances extracellular DA in the MPOA, at least in part, via nitric oxide activity. Therefore, glutamatergic stimulation of nitric oxide synthase may generate the female-induced increase in extracellular DA in the MPOA, which is important for the expression of male sexual behavior.

Key words: sexual behavior, NMDA, dopamine transporter, microdialysis.

The medial preoptic area (MPOA) of the hypothalamus is critical for the control of male sexual behavior in all vertebrate species that have been tested (reviewed in Hull et al., 2002). Although dopamine (DA) in the nucleus accumbens enhances activation for numerous motivated behaviors, including sexual behavior, DA in the MPOA is important for focusing motivation on specifically sexual activity and for coordinating genital and somatomotor responses (reviewed in Hull, 1995; Hull et al., 2002). DA is released in the MPOA as soon as a male rat detects the presence of an estrous female and during copulation (Hull et al., 1995). Both basal (Lorrain and Hull, 1993) and copulation-stimulated (Lorrain et al., 1996) DA release in the MPOA is regulated, at least in part, by nitric oxide (NO). However, factors "upstream" of NO have not been determined.

Glutamate regulates the release of DA in several brain regions both *in vitro* and *in vivo* (Whitton, 1997; Takahata and Moghaddam, 1998; Verma and Moghaddam, 1998; Shimazoe et al., 2002; Howland et al., 2002; Avshalumov et al., 2003; Katayama et al., 2003; Morikawa et al., 2003). Glutamate–DA interactions have been implicated in the regulation of various behaviors and behavioral disorders, including drug addiction (reviewed in Kelley and Berridge, 2002), schizophrenia (reviewed in Carlsson et al. 2001; Moghaddam, 2002; Pralong et al., 2002), and Parkinson's disease (reviewed in Carlsson and Carlsson, 1990; Lange et al., 1997).

The present experiments investigated the effects of exogenous glutamate on extracellular DA in the MPOA of male rats. Using *in vivo* microdialysis, experiment 1 investigated whether reverse dialysis of glutamate through the microdialysis probe would influence extracellular DA and its major metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in the MPOA of urethane-anesthetized male rats. Experiment 2 investigated whether an NO synthase (NOS) antagonist [N^G-nitro-L-arginine methyl ester (L-NAME)] or its inactive isomer [N*w*-nitro-D-arginine methyl ester (D-NAME)] would alter any glutamate-evoked effects on DA, DOPAC, and HVA.

EXPERIMENTAL PROCEDURES

Subjects

Adult male Long-Evans/Blue Spruce rats (Harlan, Indianapolis, IN, USA) were housed individually in large plastic cages. Rats were housed in a climate-controlled room, on a 14/10 h light/dark cycle, with lights off at 11:00 h and on at 21:00 h. Food and water were available *ad libitum*. All procedures were in accordance with the National Institutes of Health Guidelines for the Use of Animals, and were approved by the local Institutional Animal Care and Use Committee. All efforts were made to minimize the number of animals used and their suffering.

Surgery

Animals (approximately 300 g at the time of surgery) were anesthetized with urethane (Sigma, St. Louis, MO, USA; 1.5 g/kg) dissolved in saline. Animals received probe implants aimed at the MPOA (anterior-posterior, 2.3 mm; medio-lateral, 0.3 mm; dorsal-

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Abbreviations: aCSF, artificial cerebral spinal fluid; DA, dopamine; D-NAME, N*w*-nitro-D-arginine methyl ester; DOPAC, dihydroxyphenylacetic acid; HPLC-EC, high performance liquid chromatography with electrochemical detection; HVA, homovanillic acid; L-NAME, N^G-nitro-L-arginine methyl ester; MPOA, medial preoptic area; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; NOS, nitric oxide synthase; RM-ANOVA, repeated measures analysis of variance; TH-ir, tyrosine hydroxylase immunoreactive.

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ventral, −8.2 mm; according to Pellegrino et al., 1979). Concentric microdialysis probes were constructed in the laboratory according to the method of Yamamoto and Pehek (1990). The dialysis membrane had an outer diameter of 170 μm, an inner diameter of 150 μm, an active dialyzing length of 1 mm, and an 18,000 molecular weight cutoff. Dead volume in the outflow capillary tubing was 0.4 μl. Of the 23 probes used in experiments 1 and 2, 12 randomly picked probes exhibited *in vitro* recoveries of 26.7%±1.99, at a flow rate of 0.5 μl/min. The probe was secured to the skull and skull screws with dental acrylic.

High performance liquid chromatography–electrochemical detection (HPLC-EC)

The HPLC-EC system used to analyze all samples consisted of a Rheodyne injector (model 7520; Rohnert Park, CA, USA) with a 500 nl sample loop and an Antec (Antec-Leyden, Zoeterwoude, The Netherlands) microelectrochemical detector, equipped with a microflow cell (11 nl cell volume) with a glassy carbon working electrode and a Ag/AgCl reference electrode. The analytical column was an LC Packings (Dionex, Sunnyvale, CA, USA) Fusica reversed-phase capillary column (300 μm inner diameter, 5 cm long, packed with 3 μm C-18 particles). The working electrode was maintained at an applied potential of +0.8 V relative to the reference electrode. A Gilson Medical Electronics (Middleton, WI, USA) pump (model 307) delivered mobile phase through the system at 0.5 ml/min; however, a flow splitter divided the flow, so that flow through the analytical column was approximately 7 μl/min. The mobile phase consisted of 32 mM citric acid, 54.3 mM sodium acetate, 0.074 mM EDTA, 0.215 mM octyl sulfonic acid (Fluka, Milwaukee, WI, USA), and 4% methanol (v/v). It was filtered and degassed under vacuum; pH was 3.45. Data were collected using a Hewlett Packard computer (Hewlett-Packard, Palo Alto, CA, USA), running Gilson Medical Electronics Unipoint system controller software, which also controlled the pump parameters.

Histology

After all experimental procedures, and while the animals were still anesthetized, a dye solution was perfused through the probe to verify MPOA placement. The animals were immediately killed, and their brains were removed, frozen, and sliced (40 μm), using a cryostat. Brain slices were mounted on slides and examined for probe placement using a projection magnifier.

Statistics

The area under the curve for chromatogram peaks were averaged for the last three baseline samples. The area under the curve for each sample, including the last baseline, was then divided by the average of the three baseline samples; the resulting fraction was multiplied by 100 to yield percent change from baseline. Two-way repeated measures analyses of variance (RM-ANOVAs) were used to probe for differences in percent change from baseline for dialysate concentrations of DA, DOPAC, and HVA across samples and between groups of animals. If the two-factor analyses indicated significant differences for treatment and/or interaction, they were followed by appropriate lower-order analyses (one-factor analysis of variance for each group, Newman-Keuls post hoc comparisons between groups, and Dunnett's post hoc comparisons between the last baseline sample and each of the samples that followed).

Microdialysis

Experiment 1: reverse dialysis of glutamate or artificial cerebrospinal fluid (aCSF) alone. Immediately after surgery 14 urethane-anesthetized animals were taken to a separate test room and attached to the perfusion line. For reverse dialysis, 1 and 10 mM glutamate (Sigma) was dissolved in aCSF (in mM: 138 NaCl, 2.7 KCl, 0.5 $MgCl_2$, 1.5 KH_2PO_4, and 1.2 $CaCl_2$, pH 6.8, filtered and degassed prior to use; Sigma). Glutamate doses used were the same as those used by Youngren et al. (1993) in the nucleus accumbens. The aCSF was perfused at a constant rate of 0.5 μl/min with a KD Scientific (New Hope, PA, USA) infusion pump, using a 1-ml gastight syringe (Hamilton, Reno, NV, USA). Four to five hours after attaching the probe to the perfusion line, three baseline samples (6 min each) were collected. After collecting baseline samples from animals in the glutamate group (n=9), the perfusate was changed to aCSF with 1 mM glutamate, and five additional samples were collected. Next, perfusate was changed to aCSF with 10 mM glutamate, and five additional samples were collected. After collecting the 10 mM glutamate samples, five post-glutamate samples were collected with normal perfusate in the probe. Samples were immediately frozen (−80 °C) and later assayed using HPLC-EC. Control animals (n=5) underwent the same procedure, but received no glutamate in the perfusate. Finally, cannula placements were verified histologically, as described above.

Experiment 2: reverse dialysis of glutamate, with or without L-NAME or D-NAME. During stereotaxic surgeries, 14 animals received probe implants ending in the MPOA, similar to those in experiment 1. After collecting baseline samples, and before perfusion with glutamate, the glutamate-alone group (n=5) continued to receive perfusion with normal aCSF, while the L-NAME (n=5) and D-NAME (n=4) groups received perfusion with aCSF+5 mM L-NAME or D-NAME, respectively; during this time three additional samples were collected. Because data from experiment 1 showed a significant increase of DA in samples collected during perfusion of 10 mM glutamate, but not 1 mM glutamate, experiment 2 omitted the 1 mM glutamate perfusion. After the pretreatment samples were collected, the perfusate was changed to aCSF+10 mM glutamate for the glutamate-alone group, or aCSF+10 mM glutamate+5 mM L-NAME for the L-NAME group, or ACSF+10 mM glutamate+5 mM D-NAME for the D-NAME group.

Finally, after glutamate perfusion, three post-glutamate samples were collected with normal aCSF in the probe. (Fig. 1 describes the timeline for experiment 2.) After samples were collected, they were immediately frozen (−80 °C) and later assayed using HPLC-EC. Finally, cannula placements were verified histologically.

RESULTS

Experiment 1: reverse dialysis of glutamate or aCSF alone. Reverse dialysis of glutamate into the MPOA increased extracellular levels of DA. After exclusion of two animals with misplaced probes, analysis of data from eight animals in the glutamate group and four in the control group, with a two-factor RM-ANOVA, revealed significant differences due to treatment [$F_{(1,170)}$=7.62; P<0.05], sample [$F_{(17,170)}$=4.97; P<0.0001], and interaction [$F_{(17,170)}$=4.76; P<0.0001] (Fig. 2a). Newman-Keuls tests revealed that animals receiving 10 mM glutamate in dialysate had larger increases in extracellular DA than did control animals for 10 mM glutamate samples 2 through 5 (P<0.05). A one-factor RM-ANOVA for animals receiving glutamate revealed significant differences attributable to sample ($F_{(17,119)}$=10.2; P<0.0001); for this group, a Dunnett's t-test revealed significant increases in DA during reverse dialysis of 10 mM glutamate for samples 2 through 5 (P<0.05), compared with baseline; control animals

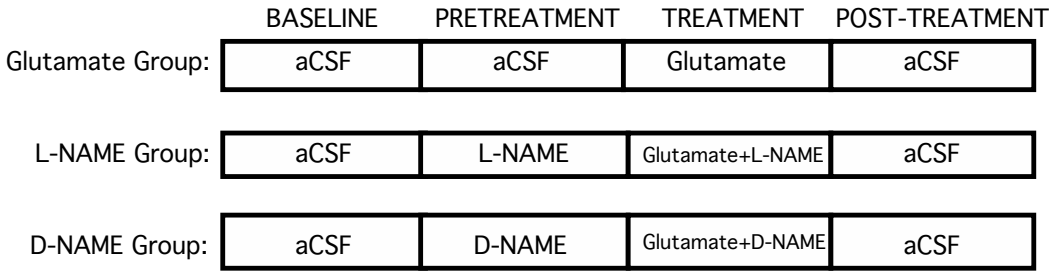

Fig. 1. Diagram showing the timeline for microdialysis in experiment 2. Following collection of baseline samples, and before perfusion with glutamate, the control group continued to receive perfusion with normal aCSF, while the L-NAME and D-NAME groups received perfusion with aCSF+5 mM L-NAME or D-NAME, respectively. After the pretreatment samples were collected, the perfusate was changed to aCSF+10 mM glutamate for the glutamate-alone group, or aCSF+10 mM glutamate+5 mM L-NAME for the L-NAME group, or ACSF+10 mM glutamate+5 mM D-NAME for the D-NAME group. Finally, post-glutamate samples were collected with normal aCSF in the probe.

showed no DA increases. A slight increase in DA during administration of 1 mM glutamate was not statistically significant. During the post-stimulation period DA levels fell and were at or slightly below basal levels by the third post-stimulation sample. We have previously reported MPOA DA basal levels in dialysate to be at 0.598±0.04 pg/μl (Dominguez et al., 2001).

Analyses of percent changes from baseline for DOPAC, with a two-factor RM-ANOVA, revealed significant differences due to treatment [$F_{(1,204)}=6.99$; $P<0.05$], sample [$F_{(17,204)}=7.08$; $P<0.0001$], and interaction [$F_{(17,204)}=3.68$; $P<0.0001$; Fig. 2b]. Newman-Keuls tests revealed that animals receiving 10 mM glutamate had lower DOPAC levels for sample 5, compared with control animals ($P<0.05$). In contrast to the effects of glutamate on DA, a one-factor RM-ANOVA for animals receiving glutamate revealed a significant decrease in levels of DOPAC, compared with baseline [$F_{(17,119)}=6.79$; $P<0.0001$]. For this group a Dunnett's *t*-test revealed decreased levels of DOPAC during perfusion with 10 mM

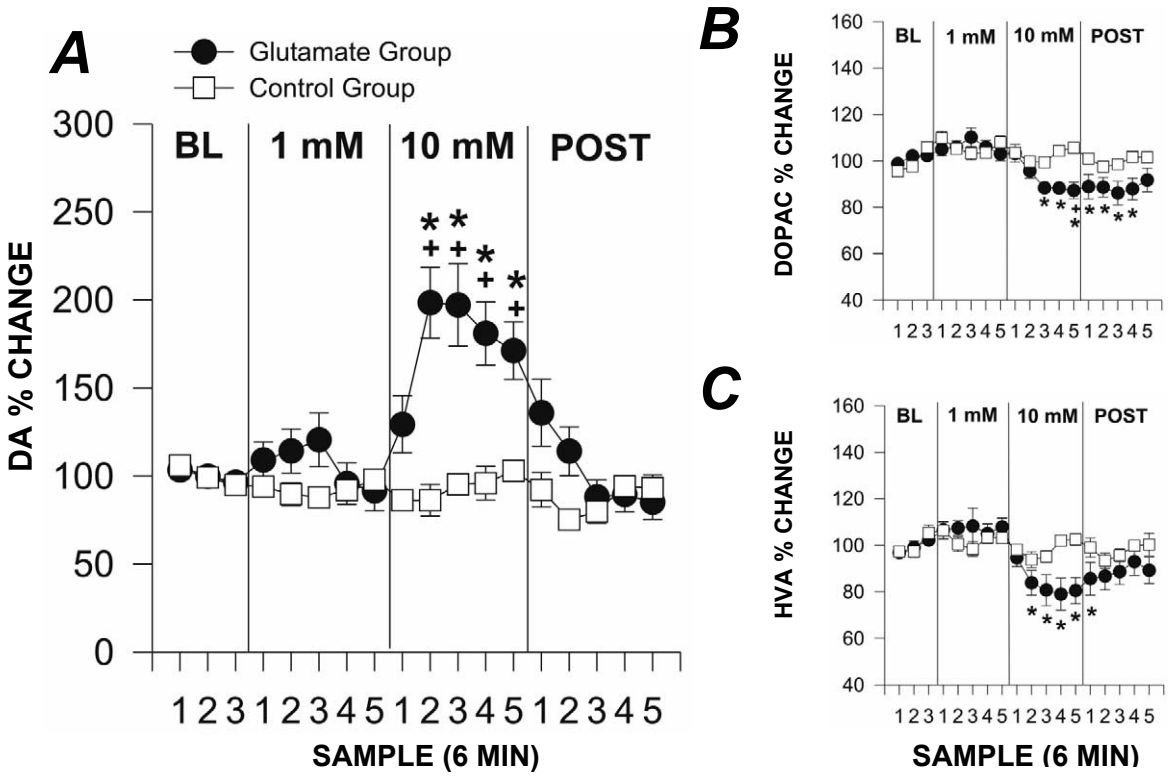

Fig. 2. (A–C) Levels of DA, DOPAC, and HVA in dialysate from the MPOA of animals receiving reverse dialysis of glutamate versus control animals. Levels represent percent changes from baseline (BL) in response to 1 mM or 10 mM glutamate in dialysate, and after removal of glutamate (POST). (A) Extracellular levels of DA significantly increased during reverse dialysis of 10 mM glutamate, compared with baseline and controls. These levels returned to baseline after glutamate was removed. (B, C) Levels of DOPAC and HVA decreased in response to 10 mM glutamate, compared with baseline. The baseline measure was obtained by dividing the value of the last baseline by the mean of all three baselines. Values are expressed as mean±S.E.M. (* $P<0.05$, compared with baseline; $^{+}$ $P<0.05$, compared with control animals).

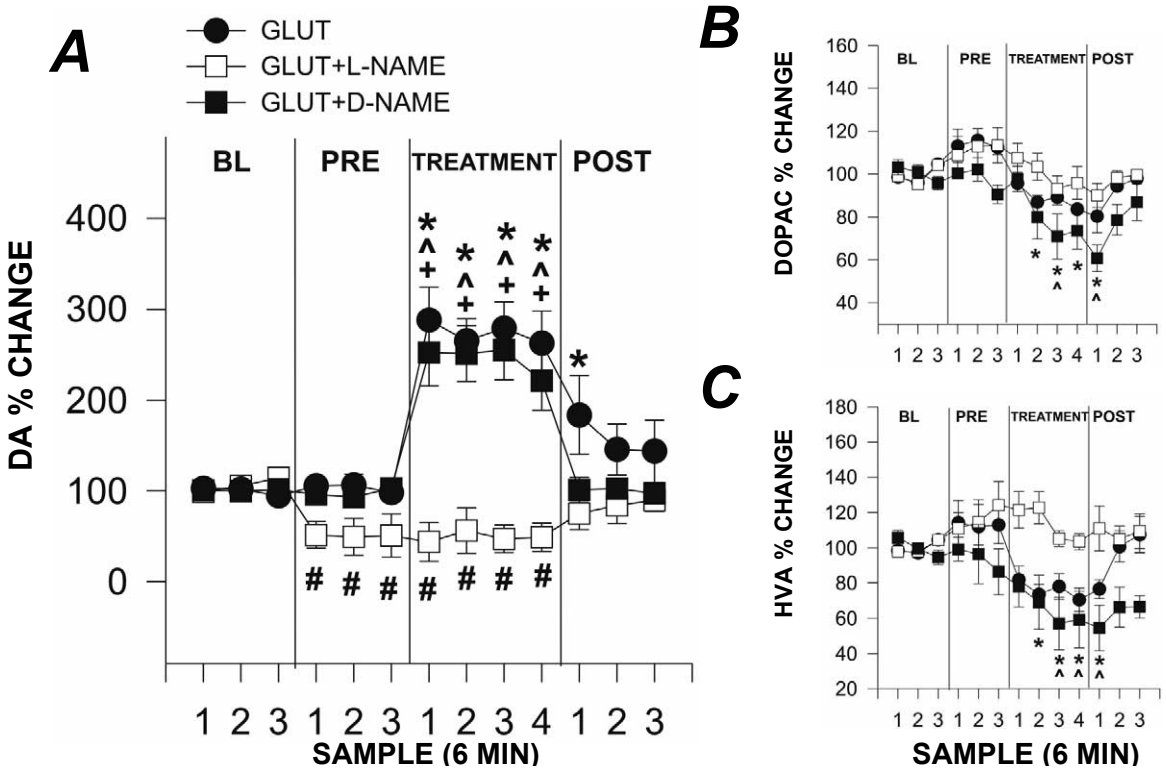

Fig. 3. (A–C) Levels of DA, DOPAC, and HVA in dialysate from the MPOA of animals receiving reverse dialysis of glutamate, glutamate+L-NAME, or glutamate+D-NAME. (A) Extracellular levels of DA significantly increased for animals receiving reverse dialysis of glutamate or glutamate+D-NAME, but not for animals receiving glutamate+L-NAME. These levels returned to baseline after glutamate was removed. Additionally, reverse dialysis of L-NAME alone decreased levels of DA, compared with baseline. (B, C) Levels of DOPAC and HVA decreased in response to glutamate and glutamate+D-NAME, but not glutamate+L-NAME, compared with baseline. The baseline measure was obtained by dividing the value of the last baseline by the mean of all three baselines. Values are expressed as mean±S.E.M. (* $P<0.05$, compared with baseline, for glutamate-alone; # $P<0.05$, compared with baseline, for L-NAME+glutamate; ^ $P<0.05$, compared with baseline, for D-NAME+glutamate; + $P<0.05$, for glutamate-alone and glutamate+D-NAME, compared with glutamate+L-NAME).

glutamate for samples 3 through 5, compared with baseline; DOPAC levels remained low for post-glutamate samples 1 through 4 ($P<0.05$). Control animals showed no significant changes in DOPAC levels.

Finally, analyses of percent changes from baseline for HVA, with a two-factor RM-ANOVA, revealed significant differences due to sample [$F_{(17,204)}=5.79$; $P<0.0001$] and interaction [$F_{(17,204)}=3.12$; $P<0.0001$], but not treatment [$F_{(1,204)}=1.69$; $P=0.217$; Fig. 2c]. A one-factor RM-ANOVA for animals receiving glutamate revealed significant differences attributable to sample [$F_{(17,119)}=6.26$; $P<0.00001$]; for this group a Dunnett's *t*-test revealed decreased levels of HVA during reverse dialysis of 10 mM glutamate for samples 2 through 5, compared with baseline; HVA levels remained low for post-glutamate sample 1, compared with baseline ($P<0.05$); control animals showed no significant changes in levels of HVA.

Experiment 2: reverse dialysis of glutamate, with or without L-NAME or D-NAME. The increase in MPOA DA observed in animals receiving glutamate via reverse dialysis was blocked by the NOS inhibitor L-NAME, but not by the inactive isomer D-NAME. After two animals with misplaced probes were excluded, four animals remained in each group (i.e. glutamate-alone, glutamate+L-NAME, and glutamate+D-NAME groups). Analyses of percent changes from baseline for DA with a two-factor RM-ANOVA revealed significant differences due to treatment [$F_{(2,108)}=11.55$; $P<0.005$], sample [$F_{(12,108)}=15.58$; $P<0.0001$], and interaction [$F_{(24,108)}=9.99$; $P<0.0001$; Fig. 3a]. Newman-Keuls tests revealed that animals receiving perfusate with 10 mM glutamate or with 10 mM glutamate+D-NAME did not differ from each other, but both had larger increases in extracellular DA than did animals receiving glutamate+L-NAME, for samples 1 through 4 ($P<0.05$). A one-factor RM-ANOVA for animals receiving glutamate alone revealed significant differences attributable to sample [$F_{(12,36)}=17.0$; $P<0.0001$]; for this group, a Dunnett's *t*-test revealed significant increases in DA for all samples collected during reverse dialysis of 10 mM glutamate and for post-glutamate sample 1 ($P<0.05$), compared with the last baseline. A one-factor RM-ANOVA for animals receiving glutamate+L-NAME revealed significant differences attributable to sample [$F_{(12,36)}=5.74$; $P<0.0001$]; for this group, a Dunnett's *t*-test revealed significant decreases in DA for all samples collected during reverse dialysis of L-NAME alone and samples 1 through 4 during glutamate+L-NAME co-administration ($P<0.05$), compared with the last baseline. Finally,

a one-factor RM-ANOVA for animals receiving glutamate+D-NAME revealed significant differences attributable to sample [$F_{(12,36)}=15.8$; $P<0.0001$]; for this group, a Dunnett's *t*-test revealed significant increases in DA for all samples collected during reverse dialysis of glutamate+D-NAME, showing that inhibition by L-NAME of the glutamate-evoked DA increase was stereospecific.

Analyses of percent changes from baseline for DOPAC, with a two-factor RM-ANOVA, revealed significant differences due to treatment [$F_{(2,90)}=6.38$; $P<0.02$] and sample [$F_{(10,90)}=13.84$; $P<0.0001$; Fig. 3b]. Despite the overall difference due to treatment, comparison of DOPAC levels among the three groups, using Newman-Keuls tests, did not reveal significant differences for any individual sample periods. However, a one-factor RM-ANOVA revealed significant differences attributable to sample for animals receiving glutamate alone [$F_{(12,36)}=8.98$; $P<0.0001$] and glutamate+D-NAME [$F_{(12,36)}=6.25$; $P<0.0001$]. For the glutamate group a Dunnett's *t*-test revealed decreased levels of DOPAC during perfusion with 10 mM glutamate for samples 2, 3, and 4, and for post-glutamate sample 1, compared with baseline ($P<0.05$). For the glutamate+D-NAME group, a Dunnett's *t*-test revealed decreased levels of DOPAC during perfusion with glutamate+D-NAME for sample 3, and for post-glutamate+D-NAME sample 1, compared with baseline ($P<0.05$). A smaller decrease in DOPAC levels in animals receiving glutamate+L-NAME was not statistically significant, compared with baseline.

Analyses of percent changes from baseline for HVA, with a two-factor RM-ANOVA, revealed significant differences due to treatment [$F_{(2,86)}=5.77$; $P<0.03$], sample [$F_{(10,86)}=8.29$; $P<0.001$], and interaction [$F_{(20,86)}=2.14$; $P<0.01$; Fig. 3c]. Despite the significant overall treatment effect, comparison of HVA levels among the three groups, using Newman-Keuls tests, did not reveal significant group differences for any sample periods. However, one-factor RM-ANOVAs revealed significant differences attributable to sample for animals receiving glutamate alone [$F_{(12,36)}=7.14$; $P<0.0001$] and those receiving glutamate+D-NAME [$F_{(12,32)}=5.74$; $P<0.0001$]. For the glutamate-alone group a Dunnett's *t*-test revealed decreased levels of HVA for samples 2, 3, and 4 collected during perfusion with 10 mM glutamate, and for post-glutamate sample 1, compared with baseline ($P<0.05$). For the glutamate+D-NAME group a Dunnett's *t*-test revealed a decreased level of HVA for samples collected during perfusion with glutamate+D-NAME samples 3 and 4, and for post-glutamate+D-NAME sample 1. A smaller decrease in animals receiving glutamate+L-NAME was not statistically significant, compared with baseline.

DISCUSSION

The present results indicate that glutamate enhances extracellular DA in the MPOA, at least in part, via NO activity. In experiment 1, exogenous glutamate, reverse dialyzed into the MPOA, increased extracellular DA levels but decreased levels of DOPAC and HVA. Levels of DA and its metabolites returned to baseline in the post-glutamate samples. To assess whether NO mediated the glutamate-evoked effects observed in experiment 1, we reverse dialyzed L-NAME or its inactive isomer D-NAME together with glutamate in experiment 2. L-NAME decreased basal DA levels and completely blocked the glutamate-evoked increase in extracellular DA and the glutamate-evoked attenuation of DOPAC and HVA levels observed in animals receiving glutamate alone. D-NAME was ineffective, indicating that L-NAME's effects were stereospecific.

Using *in vitro* and *in vivo* techniques, various studies have indicated that glutamate plays an important role in regulating DA release in the brain (reviewed in Whitton, 1997). Indeed, either glutamate receptor agonists or increased glutamate activity enhanced levels of extracellular DA in the striatum (Imperato et al., 1990a,b; Moghaddam et al., 1990; Krebs et al., 1991; Moghaddam and Gruen, 1991; Desce et al., 1992; Jones et al., 1993; Morari et al., 1993; Verma and Moghaddam, 1998; Shimazoe et al., 2002; Avshalumov et al., 2003), nucleus accumbens (Imperato et al., 1990a,b; Youngren et al., 1993; Howland et al., 2002), midbrain (Katayama et al., 2003; Morikawa et al., 2003), and prefrontal cortex (Jones et al., 1993; Jedema and Moghaddam, 1994; Takahata and Moghaddam, 1998).

Glutamate, acting via *N*-methyl-D-aspartate (NMDA) receptors, opens Ca^{2+} channels; the resultant increase in intracellular Ca^{2+} can then activate calcium calmodulin, which in turn activates NOS in some neurons. Indeed, NOS links to the carboxy-terminal tail of the NMDA receptor, via a PSD-95 protein–protein interaction domain (reviewed in Brenman and Bredt, 1997), thereby coupling NOS with the NMDA receptor. A growing body of literature (reviewed in Prast and Philippu, 2001; West et al., 2002) suggests that NO increases calcium-dependent (Lonart et al., 1993; West and Galloway, 1996, 1998; Trabace and Kendrick, 2000) and/or calcium-independent (Black et al., 1994; Meffert et al., 1996; Stewart et al., 1996) vesicular release. NO may also inhibit the DA transporter (Lonart and Zigmond, 1991; Pogun et al., 1994; Chaparro-Huerta et al., 1997; reviewed in Kiss and Vizi, 2001), thereby prolonging DA's presence in extracellular fluid. In addition, NO may increase extracellular DA indirectly by increasing the release of glutamate or via other neurotransmitter systems (reviewed in Prast and Philippu, 2001; West et al., 2002). The pattern of increased extracellular DA, together with decreased metabolites, observed in the present experiments suggests that inhibition of DA transporter activity contributed to the glutamate-induced increase in extracellular DA in the MPOA. Metabolism of DA to DOPAC requires transport into the axon terminal, where monoamine oxidase is located on mitochondrial membranes. DOPAC, in turn, may be metabolized to HVA by catechol-*O*-methyl transferase, which may be located in glia or neurons. Thus, inhibition of the DA transporter would decrease the formation of DOPAC and HVA as well as prolong DA's presence in the extracellular fluid.

The MPOA is a major integrative site for the control of male sexual behavior, and both DA and NO in the MPOA

facilitate male sexual behavior (reviewed in Hull et al., 2002). A mixed D_1/D_2 DA agonist (apomorphine), microinjected into the MPOA, facilitated copulation (Hull et al., 1986) and genital reflexes (Pehek et al., 1989), whereas a D_1/D_2 antagonist (*cis*-flupenthixol) impaired these responses and also decreased sexual motivation (Warner et al., 1991). Furthermore, stimulation of D_1 receptors in the MPOA promoted parasympathetically mediated erections, whereas stimulation of D_2 receptors shifted the autonomic balance to favor sympathetically mediated ejaculation (Hull et al., 1989, 1992). Endogenous DA was released in the MPOA as soon as male rats detected the presence of an estrous female, and levels increased further during copulation (Hull et al., 1995). MPOA DA is produced by neurons in the rostral periventricular area (A14; reviewed in Moore and Lookingland, 1995).

Normal basal levels of MPOA DA were sufficient for suboptimal copulation, but the female-stimulated increases enhanced copulatory efficiency (Dominguez et al., 2001; Putnam et al., 2003). Olfactory input, processed by the medial amygdala, is the primary stimulus for the female-induced increase in MPOA DA. Lesions of the olfactory bulbs (Nagatani and Wood, 2001) or medial amygdala (Dominguez et al., 2001) abolished the female-stimulated increase and impaired copulation, and glutamatergic stimulation of the medial amygdala elicited an increase in MPOA DA comparable to that elicited by a female (Dominguez and Hull, 2001). Furthermore, microinjection of the DA agonist apomorphine into the MPOA restored copulation to normal in males with medial amygdala lesions. There are no dopaminergic neurons in the medial amygdala; therefore, the female-stimulated increase may be mediated by glutamatergic axons, either directly from the medial amygdala or from the bed nucleus of the stria terminalis, a major relay site between the medial amygdala and the MPOA. Although extrinsic projections from the medial amygdala are largely GABAergic (Swanson and Petrovich, 1998), a few anterogradely labeled axons from the medial amygdala and numerous anterogradely labeled axons from the bed nucleus of the stria terminalis were immunoreactive for the vesicular glutamate transporter (Dominguez et al., 2003), an indicator of glutamatergic terminals. Thus, glutamatergic axons, either directly from the medial amygdala or from a major relay station between the medial amygdala and MPOA, could mediate the female-stimulated increase in MPOA DA.

Increasing NO in the MPOA also facilitated copulation (Sato et al., 1998), whereas inhibiting NOS in the MPOA impaired it (Sato et al., 1998; Lagoda et al., 2003). Furthermore, NO regulates basal levels of extracellular DA in the MPOA (Lorrain and Hull, 1993), as well as female-stimulated DA release (Lorrain et al., 1996). Reverse dialysis of a different NOS inhibitor (*N*-monomethyl L-arginine, L-NMMA) into the MPOA decreased basal extracellular DA levels and blocked the increase in DA that otherwise resulted from reverse dialysis of L-arginine, the precursor of NO (Lorrain and Hull, 1993). In another experiment L-NAME (but not D-NAME) blocked the increase in extracellular DA elicited by a receptive female (Lorrain et al., 1996). The decrease in basal levels caused by a NOS inhibitor (Lorrain and Hull, 1993) suggests that NO exerts a tonic facilitative effect on DA release and/or a tonic inhibition of the DA transporter, in addition to its acute effect demonstrated here.

NOS-immunoreactive neurons were found in the vicinity of dopaminergic (tyrosine hydroxylase immunoreactive, TH-ir) neurons, but there appeared to be no co-localization of NOS and TH in the same cells (Du and Hull, 1999). [There are no norepinephrine-containing cells in the MPOA (Simerly et al., 1986); therefore, all TH-ir neurons in the MPOA are dopaminergic.] Thus, the present experiments suggest that glutamate may mediate the female-stimulated increase in MPOA DA by increasing the production of NO, which probably diffuses from the NOS-ir neurons to the somas or axon terminals of TH-ir neurons, increasing their firing rates and/or release of DA from their terminals.

A potential criticism of this hypothesis is that the glutamate-stimulated DA increase could have resulted from excitotoxic damage to the MPOA. However, this seems unlikely for at least two reasons. First, extracellular DA returned to baseline levels in the post-stimulation samples. In contrast, extracellular DA was elevated for at least 24 h following excitotoxic damage to the striatum (Shimizu et al., 2003). Second, the MPOA appears to be unusually resistant to excitotoxic damage. For example, administration of 1 μl of 120 mM NMDA resulted in the loss of at most 10% of Nissl-stained neurons in the MPOA of male hamsters; in comparison, 1 μl of 12 mM NMDA (a 10-fold lower concentration) resulted in a massive (>90%) loss of neurons in the cortex and striatum (Ebling et al., 1998). The reason for the resistance of the MPOA to excitotoxicity is not clear, as expression of NMDAR1 and NMDAR2 subunits was comparable for the MPOA, striatum, and cortex (Ebling et al., 1998). It is likely that differential linkage to signal transduction pathways in these brain areas mediates the differential sensitivity (Michaelis, 1998).

In summary, glutamate, reverse dialyzed into the MPOA, increased extracellular levels of DA but decreased extracellular levels of DA metabolites, suggesting that the DA transporter was inhibited. DA levels returned to baseline during the post-glutamate samples. In addition, reverse dialysis of a NOS inhibitor, L-NAME, but not its inactive isomer, D-NAME, blocked glutamate's effects. Therefore, glutamatergic activity in the MPOA, acting via NO, could underlie the female-stimulated increase in DA concentrations, which in turn facilitates male sexual behavior.

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