Life Sciences, Vol. 52, pp. 935-940 Pergamon Press
Printed in the USA

COPULATION INCREASES DOPAMINE ACTIVITY IN THE MEDIAL PREOPTIC AREA OF MALE RATS

Elaine M. Hull, Robert C. Eaton, Jason Moses, and Daniel Lorrain

Department of Psychology, State University of New York at Buffalo, Buffalo, NY 14260

(Received in final form December 28, 1992)

Summary

Dopamine (DA) metabolites in microdialysates from the medial preoptic area (MPOA) of male rats increased during copulation. These increases were not observed during eating of a highly palatable food, or if the animal failed to copulate, or if the microdialysis probe was anterior or dorsal to the MPOA. The only two animals with measurable serotonin (5-HT) levels while the female was present were also the only two that either failed to copulate or copulated but failed to ejaculate. These data are consistent with previous evidence for a facilitative role of MPOA DA in the control of male sexual behavior; however, 5-HT activity in the MPOA may impair copulation.

Dopaminergic activity in either the nucleus accumbens (NAcc) or medial preoptic area (MPOA) appears to facilitate male copulatory behavior. Injection of the dopamine (DA) agonists amphetamine (1) or apomorphine (2) into the NAcc speeded the onset of copulation, and injection of apomorphine into the MPOA increased copulatory rate (2). Endogenous DA was released in the NAcc of male rats as soon as a receptive female was presented and/or the two were allowed to copulate (3-7). However, similar increases of DA release in the NAcc have been observed with feeding and reinforcing drug or electrical stimulation (e.g., 8-13). Therefore DA activity in the NAcc has been suggested to promote a state of general incentive motivation, rather than specifically activating any particular motivated behavior (e.g., 1,3,14-16).

Catecholamine release in the MPOA, measured with in vivo voltammetry, showed a pattern of release before and during copulation similar to that in the NAcc (17). However, it was not clear whether the catecholamine signal was due to DA or norepinephrine (NE) release, or if it was specific to copulatory behavior. The present experiment used microdialysis in the MPOA to measure the release of DA and its major metabolites DOPAC and HVA as well as of 5-HT and its metabolite 5-HIAA. Samples were collected before, during, and after copulation and/or feeding in order to determine the behavioral specificity of neurotransmitter release.

Methods

Animals

Twelve sexually experienced male Long-Evans rats (Harlan-Sprague Dawley/Blue Spruce Farms, Altamont, NY) 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 lib.

Corresponding author: Elaine M. Hull, Department of Psychology, Park Hall, State University of New York at Buffalo, Buffalo, NY 14260.

0024-3205/93 $6.00 + .00

Surgery
The male rats were anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4 mg/kg). Each animal received a 21 ga thin wall unilateral stainless steel guide cannula ending above the MPOA [AP, +2.4; ML, +0.2; DV, -7.0; incisor bar, + 5 mm, (18)]. Details of the surgery and cannula construction are described in (2). 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 27 ga stainless steel tubing and the cap of the centrifuge tube, was cut to end even with the guide cannula.

Microdialysis
Microdialysis probes using a concentric flow design were made according the procedure of Yamamoto and Pehek (19). A length of silicon capillary tubing (150 μm o.d.) was inserted into a piece of 26 ga stainless steel tubing extending approximately 1 mm beyond the tip of the stainless steel tube. A hollow fiber dialysis membrane (MW cutoff = 6000; 210 μm o.d.; Spectrum; Spectra/Por) was fitted over the silicon capillary tube and glued to the tip of the stainless steel tubing. 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.

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 7.4). Perfusion flow was controlled by a Harvard syringe infusion pump (Model 22) at a rate of 0.75 μl/min. Dialysates were collected every 40 min.

On the day of testing, the animal was briefly anesthesized with Metofane 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 hr stabilization period occurred prior to collecting baseline samples. Overhead lights were turned off at 11.00 hr (during the 2 hr stabilization period), and the experiment was completed using dim illumination. At least three baseline samples were collected, until levels were stable (no more than 10% variation). Four animals received food (a peanut butter filled cookie) during one sample preceding copulation tests; two additional animals received food following copulation; the remaining six animals received only copulation tests. Two washout samples separated the copulation and feeding tests. For copulation samples a sexually receptive female was placed into the testing chamber for 80 min (2 collection intervals) and copulatory activity was monitored. The female was removed from the chamber and collection of dialysates continued for 40 min. Rats were anesthetized and decapitated; the brains were frozen, and the probe placements were histologically verified.

Chromatography
Ascorbate oxidase (2 μl) was added to the dialysate sample (30 μl) and kept in the dark for 5 min prior to injection. Samples were assayed for the parent amine DA and its metabolites DOPAC and HVA and for serotonin (5-HT) and its metabolite 5-HIAA using high performance liquid chromatography with electrochemical detection (HPLC-EC). Compounds were separated on an Ultracarb 3 μm C18 column (100 mm in length) 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 0.5% methanol (pH = 4.5). A BAS model PM-48 pump, equiped with a flow splitter and pulse damper (SSI), operated at a flow rate of 1.4 ml/min. Compounds were detected with a BAS LC-4B amperometric detector (sensitivity = 5 nA/V), using a glassy carbon electrode maintained at a potential of +0.7 V relative to a Ag/AgCl reference electrode. Standards for NE, DA, and 5-HT (10 pg/20 μl), and for DOPAC, 5-HIAA, and HVA (100 pg/20 μl) were prepared weekly from stock solutions made with compounds from Sigma Chemical. Vehicle for the standards was Dulbecco's phosphate buffered saline; pH was 7.4. The limit of detectability (3 x background) was .5 pg/20 μl.

Data Analysis

The data are expressed as a percentage of the average of the three baseline samples preceding a behavioral test. For statistical comparisons, the last of the three baseline samples was also expressed as a percentage of the average baseline. Analysis of the data was by ANOVA followed by Newman-Keuls pair-wise comparisons. Only animals that had verfied probe placement in the MPOA were included in the analysis.

Results

Extracellular levels of the DA metabolites DOPAC and HVA were elevated in all seven animals that copulated and had accurately placed microdialysis probes (DOPAC: $F(3,18)=6.59$, $p<.004$; HVA: $F(3,18)=10.69$, $p<.0005$; see Fig. 1). These increases were observed during both copulation samples and the following washout sample. In five of the seven animals, DA itself also increased during at least one of the two copulation samples; in the two remaining animals DA was below the limit of detectability. The increase in DA was not statistically significant in comparisons of each interval in all seven animals. DA metabolites failed to rise in four other animals that had probes located anterior or dorsal to the MPOA, and in one animal that had a correctly placed probe but failed to copulate. Feeding failed to elicit a consistent release of any neurotransmitter or metabolite. Thus, the increased DA activity was localized and was dependent upon copulation.

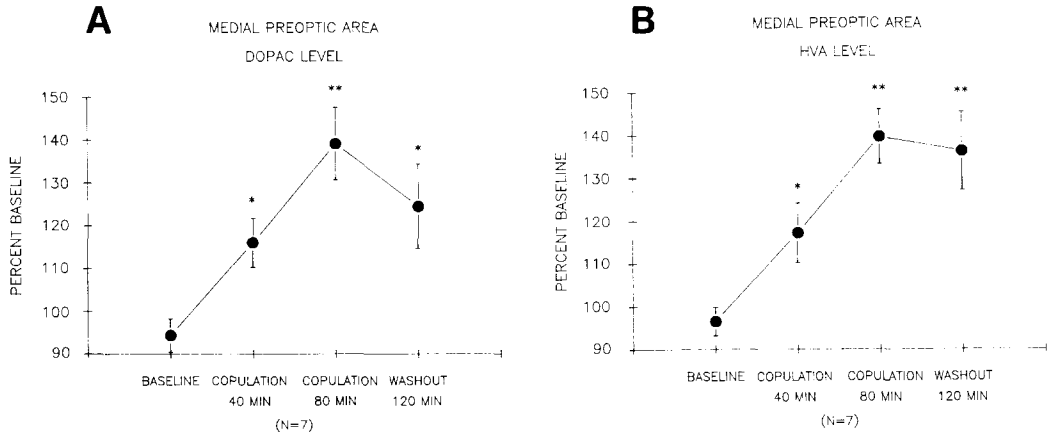

Fig. 1

Effects of copulation on extracellular DOPAC and HVA in the MPOA. Both metabolites were increased during 80 min of copulation and in the subsequent 40 min washout period. Values are mean percent baseline ± SEM. **$p<.01$, *$p<.05$, relative to baseline.

In all but two animals 5-HT declined to nondetectable levels during baseline samples and remained below the limit of detectability during the remainder of the experiment. In one of the two aberrant animals 5-HT rose in the second feeding sample and remained high after introduction of the female; that animal was the only one that failed to copulate and the only one with a correctly placed probe that failed to show an increase in DOPAC and HVA. The second aberrant animal had an elevation of 5-HT in the fifth baseline sample; 5-HT subsequently declined, but remained detectable during the first copulatory sample. This animal exhibited numerous mounts, but few intromissions and no ejaculations; he did show an increase in DOPAC and HVA similar to the increases seen in other copulating animals.

Discussion

MPOA dopamine activity showed consistent elevations during copulation. These increases were site specific and were not observed when the animals ate a highly palatable food. These data are consistent with a previous observation, using in vivo voltammetry, of catecholamine release in the MPOA during copulation (17). It appears that the catecholamine signal in that study probably reflected DA activity, at least in part, since DA itself increased during copulation in a majority of the animals in the present study. (NE was undetectable in the present experiment because it eluted in the solvent front.) Noradrenergic neurons do not contribute to basal DOPAC levels in tissue punches from the MPOA (20); however, a contribution of noradrenergic neurons to copulation-induced DOPAC increases cannot be ruled out. Additional studies are planned to address this question.

DA activity in the MPOA may be more specifically associated with copulation than is DA activity in the NAcc, which seems to be related to a generalized motivational state. Eating a highly palatable food did not increase DA activity in the MPOA, whereas numerous incentives increase DA activity in the NAcc (e.g., 1,3,14-16). On the other hand, Hoffman et al. (21) reported that both copulation and forced running on a treadmill increased DA in MPOA tissue punches from animals killed after either copulatory exhaustion or a matched length of time of enforced running on a treadmill. Thus, while there is some behavioral specificity to the increased DA activity in the MPOA, these increases are not entirely selective.

Mas et al. (22) analyzed MPOA tissue punches from animals killed after either no copulation, one intromission, or one ejaculation. DOPAC was elevated after one intromission and after one ejaculation. However, DA was significantly elevated only after ejaculation. It is not clear whether the increase in DA after ejaculation was specific to ejaculation, or whether it resulted from the additional copulation required to achieve ejaculation. The one animal in the present study that mounted and intromitted, but failed to ejaculate, did not show an increase in extracellular DA. However, it is difficult to compare the present data with those of Mas et al., because amines in tissue samples represent primarily intracellular stores, whereas those in microdialysate have been released.

Because increased DA activity has been observed in several brain areas during copulation and/or other behaviors, there has been speculation about the function of this activity. DA appears to facilitate the functions of the areas it innervates, rather than being part of the integral circuitry of those areas (see 23,24). Thus, sustained DA release may enhance specific sensory input and facilitate the linkage with specific outputs. In addition, rising and/or prolonged DA release may sequentially activate neural processes with different thresholds.

Several experiments support a facilitative role for DA in the MPOA for male sexual behavior. Exogenous stimulation of DA receptors in the MPOA facilitated copulation (2) and ex copula penile reflexes (25), whereas blocking DA receptors there impaired copulation (26) and decreased sexual motivation and ex copula penile reflexes (27). Similarly, 6-OHDA lesions of DA terminals in the MPOA impaired copulation in tests within 4 hrs of the lesions (28), or after inhibition of the compensatory increase in DA synthesis (28,29). Thus, exogenous stimulation of DA receptors in the MPOA facilitates sexual behavior, and blocking endogenous DA's access to those receptors impairs it, as does removing DA terminals.

Rising and/or prolonged release of DA in the MPOA may also shift from primarily parasympathetically controlled erections to sympathetically elicited ejaculation. A low dose of apomorphine in the MPOA increased ex copula penile erections and anteroflexions via D1 receptors, and a high dose increased seminal emissions via D2 receptors (30). Thus, low levels of DA stimulation in the MPOA may enhance erectile processes, whereas high levels of DA stimulation may activate ejaculatory mechanisms.

On the other hand, elevated 5-HT in the MPOA may interfere with copulation. The only two animals with measurable 5-HT while the female was present showed either no copulation or

copulation without ejaculation. Mas et al. (22) reported that 5-HIAA levels in ex vivo MPOA tissue punches increased after ejaculation but not after a single intromission. They suggested that elevated 5-HT synthesis in the MPOA during the postejaculatory interval may inhibit copulation at that time. Numerous studies have reported inhibitory effects of 5-HT on male sexual behavior; these effects have recently been attributed to 5-HT1B (31) or 5-HT2 (32) receptors. Also, injections of 5-HT into the NAcc inhibited copulation (33). However, 5-HT1A agonists injected into the MPOA (34), the medial raphe or NAcc (33), or systemically (e.g., 35,36) have facilitated male sexual behavior. Thus, 5-HT has complex influences on masculine sexual behavior.

In summary, DA activity in the MPOA increased during copulation, but not during eating of a highly palatable food. This increase was not observed in animals whose microdialysis probes were located anterior or dorsal to the MPOA. These data are consistent with a facilitative role for DA in the MPOA in the control of masculine sexual behavior.

Acknowledgements

We are extremely grateful to Dr. Elizabeth A. Pehek and Dr. Bryan K. Yamamoto for demonstrating their techniques for microdialysis, HPLC-EC, and probe construction and for much valuable advice throughout this project. We also thank Edward C. Werts for laboratory assistance. This work was supported by NIMH grant MH-40826 and BRSG grant 2SO7RR0706626 to EMH.

References

1. B.J. EVERITT, Neurosci. Biobehav. Rev. 14 217-232 (1990).
2. E.M. HULL, D. BITRAN, E.A. PEHEK, R.K. WARNER, L.C. BAND, & G.M. HOLMES, Brain Res. 370 73-81 (1986).
3. G. DAMSMA, J.G. PFAUS, D. WENKSTERN, and A.G. PHILLIPS, Behav. Neurosci. 106 181-191 (1992).
4. A. LOUILOT, J.L. GONZALEZ-MORA, T. GUADALUPE and M. MAS, Brain Res., 553 313 (1991).
5. M. MAS, J. GONZALEZ-MORA, A. LOUILOT, C. SOLE, and T. GUADALUPE, Neurosci. Lett., 110 303-308 (1990).
6. J.G. PFAUS, G. DAMSMA, G.G. NOMIKOS, D. WENKSTERN, C.D. BLAHA, A.G. PHILLIPS, and H.C. FIBIGER, Brain Res. 530 345-348 (1990).
7. E.T. PLEIM, J.A. MATOCHIK, R.J. BARFIELD, and S.B. AUERBACH, Brain Res. 524 160-163 (1990).
8. J. CHEN, W. PAREDES, J.H. LOWINSON, and E.L. GARNDER, Neurosci. Lett. 129 136-140 (1991).
9. G. DI CHIARA, E. ACQUAS, and E. CARBONI, The Mesolimbic Dopamine System: From Motivation to Action, P. Willner and J. Scheel-Kruger (Eds), 367-384, Wiley and Sons, Chichester (1991).
10. L. HERNANDEZ, N.A. GUZMAN, and B.G. HOEBEL, Psychopharmacol. 105 264-268 (1991).
11. G.P. MARK, D.H. SCHWARTZ, L. HERNANDEZ, H.L. WEST and B.G. HOEBEL, Microdialysis in the Neurosciences, T.E. Robinson and J.B. Justice Jr. (Eds), 369-385, Elsevier Press, Amsterdam (1991).
12. D. NAKAHARA, K. FUCHIKAMI, N. OZAKI, T. IWASAKI and T. NAGATSU, Brain Res., 574 164-170 (1992).
13. F.S. RADHAKISHUN, J.M. VAN REE, B.H.C. WESTERINK, Neurosci. Lett., 85 351-356 (1988).
14. G.J. MOGENSON, D.L. JONES and C.Y. YIM, Progr. Neurobiol., 14 69-97 (1980).
15. H.C. FIBIGER and A.G. PHILLIPS, The Mesocorticolimbic Dopamine System, P.W. Kalivas and C.B. Nemeroff (Eds), 206-215, New York Academy of Sciences, NY (1988).
16. R.A. WISE and M.A. BOZARTH, Brain Res. Bull., 12 203-208 (1984).
17. J.G. BLACKBURN, J.G. PFAUS, and A.G. PHILLIPS, Prog. Neurobiol., 39 247-279 (1992).

18. L.J. PELLEGRINO, A.S. PELLEGRINO, and A.J. CUSHMAN, A Stereotaxic Atlas of the Rat Brain, 2nd ed. Plenum Press, New York, (1979).
19. B.K. YAMAMOTO and E.A. PEHEK, Brain Res. 506 236-241 (1990).
20. K.J. LOOKINGLAND, D.S. CHAPIN, D.W. McKAY, and K.E. MOORE, Brain Res. 365 228-234 (1986).
21. N.W. HOFFMAN, A. GERALL, and P.W. KALIVAS, Physiol. Behav. 41 563-569 (1987).
22. M. MAS, A.R. DEL CASTILLO, M. GUERRA, J.M. DAVIDSON, and E. BATTANER, Physiol. Behav. 41 341-345 (1987).
23. B.S. BUNNEY, L.A. CHIODO, and A.A. GRACE, Synapse, 9 79-94 (1991).
24. H. SIMON and M. LE MOAL, The Mesocorticolimbic Dopamine System, P.W. Kalivas and C.B. Nemeroff (Eds), 206-215, New York Academy of Sciences, New York (1988).
25. E.A. PEHEK, J.T. THOMPSON, and E. HULL, Brain Res., 500 325-332 (1989).
26. E.A. PEHEK, R.K. WARNER, T.J. BAZZETT, D. BITRAN, L.C. BAND, R.C. EATON, and E.M. HULL, Brain Res., 443 70-76 (1988).
27. R.K. WARNER, J.T. THOMPSON, V.P. MARKOWSKI, J.A. LOUCKS, T.J. BAZZETT, R.C. EATON, and E.M. HULL, Brain Res., 540 177-182 (1991).
28. T.J. BAZZETT, L.A. LUMLEY, D. BITRAN, V.P. MARKOWSKI, R.K. WARNER and E.M. HULL, Brain Res., 580 164-171 (1992).
29. D. BITRAN, E.M. HULL, G.M. HOLMES and K.J. LOOKINGLAND, Brain Res. Bull., 20 323-331 (1988).
30. E.M. HULL, R.C. EATON, V.P. MARKOWSKI, J. MOSES, L.A. LUMLEY, and J.A. LOUCKS, Life Sci., 51 1705-1713 (1992).
31. A. FERNANDEZ-GUASTI, A. ESCALANTE and A. AGMO, Pharmacol. Biochem. Behav. 34 811-816 (1989).
32. M.M. FOREMAN, J.L. HALL and R.L. LOVE, Life Sciences, 45 1263-1270, 1989.
33. V. HILLEGAART, S. AHLENIUS, and K. LARSSON, Behav. Brain Res., 42 169-180 (1991).
34. L.A. LUMLEY, J. MOSES, R.C. EATON and E.M. HULL, Soc. Neurosci. Abst., 18 537 (1992).
35. S. AHLENIUS and K. LARSSON, Behavioral Pharmacology of 5-HT, P. Bevan, A.R. Cools and T. Archer (Eds), 35-53, Lawrence Erlbaum, Hillsdale (1989).
36. L.L. KWONG, E.R. SMITH, J.M. DAVIDSON, and S.J. PEROUTKA, Behav. Neurosci. 100 664-668 (1986).