ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 1997, 57: 283-287

Nitro-l-arginine attenuates SKF 38393 - induced oral activity in neonatal 6-hydroxydopamine--lesioned rats

Ryszard Brus[1], Ryszard Szkilnik[1], Przemysław Nowak[1] and Richard M. Kostrzewa[2]

[1]Department of Pharmacology, Silesian Academy of Medicine, 38 H. Jordan St., 41-808 Zabrze, Poland; [2]Department of Pharmacology, Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37614, USA

Abstract. Nitric oxide (NO) in brain has been implicated in neuronal regulatory processes and in neuropathologies. Previously we showed that NO modified quinpirole-induced yawning, a behavioral measure of dopamine (DA) D_3 receptor activation in rats. The aim of this study was to characterize the effect of nitro-L-arginine methyl ester HCl (NAME) and L-arginine HCl on reactivity of rats to the DA D_1 receptor agonist SKF 38393 and DA D_1 antagonist SCH 23390 in intact and neonatal 6-hydroxydopamine (6-OHDA)-lesioned rats (134 μg of base ICV at 3rd day after birth). L-arginine HCl (300 mg/kg IP) increased the oral activity response in 6-OHDA-lesioned rats, like SKF 38393, and induced catalepsy in intact control rats, like SCH 23390. In contrast, NAME had no effect on oral activity or catalepsy, but fully attenuated SKF 38393-induced oral activity. These findings indicate that L-arginine HCl has no apparent effect at the DA D_1 receptor, but that NAME is effective in attenuating a DA D_1 agonist - induced effect. Consequently NO may be an intracellular second messenger for supersensitized receptors associated with DA D_1 agonist - induced oral activity.

Key words: nitric oxide, dopamine D_1 receptors, 6-OHDA, central nervous system, rats, oral activity, catalepsy

INTRODUCTION

Previously we showed that the nitric oxide (NO)-donor, L-arginine HCl (ARG), and NO-synthase inhibitor, L-N-nitro-L-arginine methyl ester HCl (NAME), had opposite effects on quinpirole - induced yawning (Brus et al. 1996) a behavioral measure of DA D_3 receptors activation (Damsma et al. 1993). The aim of this study was to examine the role of ARG and NAME on reactivity of central DA D_1 receptors to the DA D_1 agonist 1-phenyl-2,3,4-5-tetrahydro-1H-3-benzazepine-7,8-di ol (SKF 38393) and DA D_1 antagonist R-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-be nzazepine (SCH 23390). Because D_1 receptors are supersensitized in rats lesioned neonatally with 6-hydroxydopamine (6-OHDA), both intact and 6-OHDA-lesioned rats were behaviorally assessed for oral activity and catalepsy - actions known to be produced by SKF 38393 and SCH 23390, respectively.

METHODS

Wistar albino rats were bred in a home colony and housed at 22 ± 1°C on a light /dark 12 h cycle (light on at 07 00 h). Rats had free access to rodent food pellets (Altromin - 1324, Lage, Germany) and water. At 3 days after birth animals were pretreated with desipramine HCl (20.0 mg/kg IP, base form; Sigma Chemical Co., St. Louis, MO), 1 h before bilateral intracerebroventricular (ICV) injection of 6-OHDA HBr (66.7 µg base on each side; Sigma Chemical Co., St. Louis, MO) or saline-ascorbic acid (0.1%) vehicle. This procedure has been described in detail (Kostrzewa and Gong 1991). Rats were weaned at 21 days. Behavioral experiments were performed on male rats, starting about 3 months after birth.

For assessing oral activity rats were placed in individual clear glass chambers in a quiet, well-ventilated and well-lighted room. After an acclimation period of at least 30 min, saline vehicle 1.0 ml/kg or SKF 38393 HCl in a dose 0.03, 0.1, 0.3 or 1.0 mg/kg was injected IP. Each rat was then observed one at a time, for 1 min every 10 min, over a 60 min period, beginning 10 min after the last treatment. Numbers of vacuous chewing movements were counted by an experienced observer. Each rat was tested only once a day, and the results were expressed in the form of dose-response curves. The observed maximal SKF 38393 effect in 6-OHDA-lesioned rats was 30 to 40 vacuous chewing movements (Brus et al. 1994). Oral activity in these studies is of the type described in the paper by Waddington (1990), as spontaneous chewing movements that are not directed toward any physical material.

ARG HCl (300 mg/kg; Sigma Chemical Co., St. Louis, MO), NAME (25 mg/kg; Sigma Chemical Co., St. Louis, MO) or vehicle was administered IP 10 min before a single IP injection of SKF 38393 0.1 mg/kg or vehicle. Numbers of vacuous chewing movements were counted as previously.

The inclined screen procedure of Iorio et al. (1986) was used to assess SCH 23390-induced catalepsy, as follows: ARG 300 mg/kg, NAME 25 mg/kg or saline vehicle 1.0 ml/kg was administered IP 10 min before SCH 23390 HCl 0.5 mg/kg or saline vehicle IP injection. Thirty minutes later rats were placed in the center of a 25 x 50 cm wire mesh screen (1 cm squares) inclined at 60° from horizontal. The time (in s) taken for each rat to move any paw at least one screen division was recorded (up to 60 s). The sum of three measurements taken at 30-min intervals was defined as catalepsy time.

At the conclusion of both behavioral studies, rats were decapitated and brains were dissected. Striatal concentrations of DA and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) were analyzed by liquid chromatography with electrochemical detection (HPLC/EC, Gilson).

Differences between groups were determined by an analysis of variance (ANOVA), followed by post-ANOVA test of Neuman-Keuls.

RESULTS

In rats lesioned as neonates with 6-OHDA, the striatal content of DA was reduced in adulthood by about 95%, from 71.1 ± 4.1 (n = 7) in controls to 3.3 ± 0.4 (n = 8) nmol/g ($P < 0.001$), and striatal content of DOPAC was reduced from 24.7 ± 1.9 (n = 7) in controls to 0.52 ± 0.10 (n = 8) nmol/g ($P < 0.001$).

Challenge doses of SKF 38393 HCl (0.03 to 1.0 mg/kg IP) increased numbers of oral movements in 6-OHDA-lesioned but not in control rats (Fig. 1). At an optimal dose of SKF 38393 HCl (0.1 mg/kg IP) 6-OHDA-lesioned rats had 30 chewing movements during the 60 min observation period. This is comparable in magnitude to other studies involving SKF 38393-induced oral activity in 6-OHDA-lesioned rats (Kostrzewa and Gong 1991, Brus et al. 1994).

ARG (300 mg/kg IP) alone produced an increase in oral activity of 6-OHDA-lesioned rats and did not alter

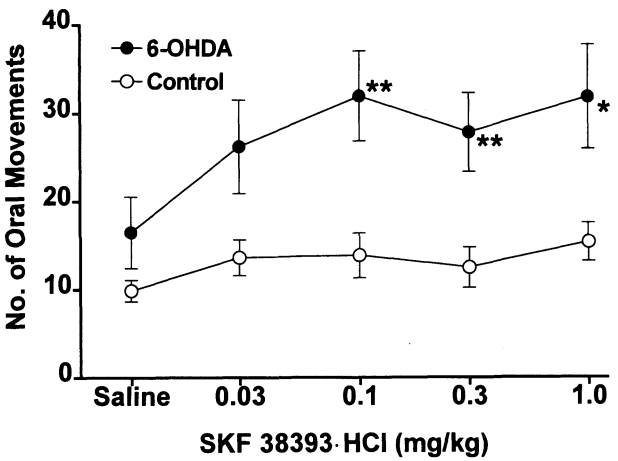

Fig. 1. Effect of SKF 38393 on numbers of oral movements of control (neonatal saline- ascorbic acid, 0.1%; 5 µl ICV on each side) and neonatal 6 OHDA-lesioned rats (66.7 µg of 6-OHDA base, ICV, on each side). Each group is the mean (± SEM) of 8 rats. * $P<0.05$, ** $P<0.01$, control group vs. 6-OHDA-lesioned group at the same SKF 38393 dose.

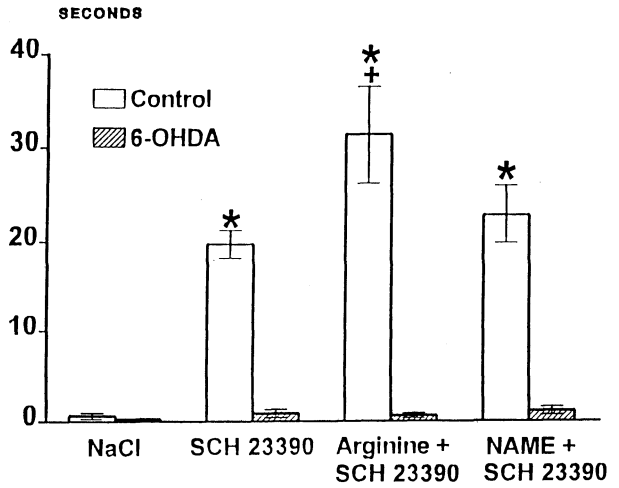

Fig. 3. Effects of arginine and NAME on SCH 23390-induced catalepsy in intact and neonatal 6-OHDA-lesioned rats. Ten minutes before SCH 23390 HCl (0.5 mg/kg IP), rats were injected IP with L-arginine HCl (300 mg/kg), L-NAME (25 mg/kg), or vehicle. Catalepsy time represents the cumulative time (in sec) in three sessions (30 min intervals) for a rat to move a paw at least one screen division on a wire mesh screen (25 x 50 cm, 1 cm squares) inclined at 60° from horizontal. Catalepsy was recorded, starting 30 min after SCH 23390 or NaCl vehicle. Maximum catalepsy time is 180 s (60 s x 3). Each group is the mean of 8 rats. * $P<0.005$ vs. NaCl effect in the same group of rats; + $P<0.05$ vs. SCH 3390 alone in the same group of rats.

the oral activity response to SKF 38393 (Fig. 2). In contrast, NAME alone (25 mg/kg IP) reduced the number of oral movements in intact rats and attenuated SKF 38393-induced oral activity in lesioned rats (Fig. 2).

Neither NAME alone nor ARG alone altered catalepsy time in intact and 6-OHDA - lesioned rats (not shown). The DA D1 receptor antagonist SCH 23390 substantially increased catalepsy time in intact rats and was ineffective in 6-OHDA-lesioned rats, as expected (Fig. 3). Although NAME (25 mg/kg IP) was without effect on SCH 23390-induced catalepsy, ARG pretreatment (300 mg/kg IP) increased the catalepsy time of SCH 23390 in intact rats. ARG and NAME, like SCH 23390, did not induce catalepsy in 6-OHDA-lesioned rats.

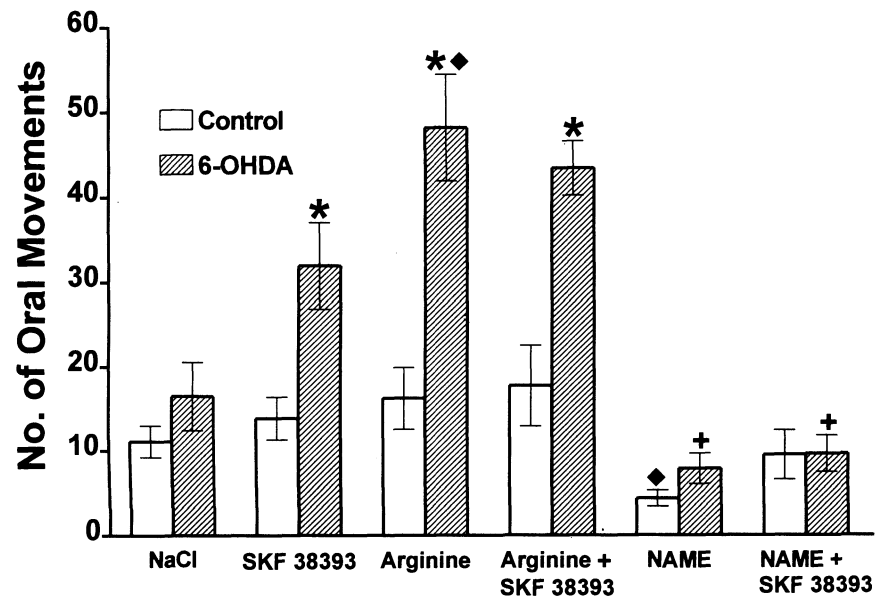

Fig. 2. Effects of arginine and NAME on SKF 38393-induced oral activity of control and neonatal 6-OHDA-lesioned rats. Ten minutes before SKF 38393 HCl (0.1 mg/kg IP), rats were injected IP with L-arginine HCl (300 mg/kg), L-NAME (25 mg/kg), or vehicle. Oral activity was observed for 60 min, starting 10 min after the last injection. Each group is the mean (± SEM) of 8 rats. *$P<0.01$, vs. control rats with some challenge substance. ♦ $P<0.01$, vs. NaCl effect in some group of rats. + $P<0.01$, vs. SKF 38393 effect in lesioned rats.

DISCUSSION

The DA D_1 complex receptors (D_1, D_5) and D_2 complex receptors (D_2, D_3, D_4) each can display enhanced sensitivity (reactivity) and density (numbers), and associated changes in equilibrium status (Sokoloff et al. 1993). All of these elements are implicated in abnormal animal behavior and in human neurological and psychiatric disorders. An increase in the reactivity of D_2 receptors is thought to occur in schizophrenia, while an increase in the reactivity of both D_1 and D_2 receptors is thought to occur in Parkinsons disease and Huntingtons chorea (Calne 1984, Barone et al. 1986, Koller and Herbster 1988).

The DA D_1 agonist SKF 38393 is known to induce oral activity in intact rats (Rosengarten et al. 1983, 1986) and to have enhanced effect in rats DA-denervated in ontogeny with 6-OHDA (Kostrzewa and Gong 1991, Kostrzewa and Hamdi 1991, Brus et al. 1994). A change in second messenger production is suspect as a mechanism underlying DA receptor supersensitivity, because binding indices of DA receptors (i.e., B_{max} and K_d) are unaltered when DA supersensitivity is produced (Breese et al. 1987, Hamdi and Kostrzewa 1991, Brus et al. 1994). The current study replicates previous findings on SKF 38393 and extends this by showing that NO modification alters reactivity of D1 receptors.

In the current study the NO donor ARG induced oral activity in DA-lesioned rats and the effect was not enhanced by co-treatment with SKF 38393. The NO synthase inhibitor NAME had little effect alone, but completely attenuated SKF 38393-induced oral activity. In one study (Pogun et al. 1994) but not in others (Zhu and Luo 1992, Lonart et al. 1993) NO inhibited DA release in the striatum of rats, and inhibited DA uptake into striatal synaptosomes without altering amphetamine-induced DA release in the nucleus accumbens *in vitro* (Pogun et al. 1994). Other studies similarly implicate NO in DA neurotransmission in striatum (Lin et al. 1995). Previously, we found that NO modulated quinpirole-induced yawning behavior, an indicator of central DA D_3 receptor activation (Brus et al. 1996). Others have found that peripheral and central administration of NAME and N-monomethyl-L-arginine (NMMA), another NO synthase inhibitor, prevents apomorphine-induced yawning behavior in rats (Melis and Argiolas 1993).

The major finding in the current study is that the NO synthase inhibitor NAME, while lacking an effect alone, potently attenuated SKF 38393-induced oral activity in intact and in 6-OHDA-lesioned rats. Because SKF 38393 induces oral activity in rats *via* an action of DA D_1 receptors, it appears that NAME attenuated reactivity or sensitivity of DA D_1 receptors. Because NAME did not have an effect of its own on catalepsy, nor alter catalepsy time following administration of the DA D_1 receptor antagonist SCH 23390, the effect of NAME at the D_1 receptor was apparently related to agonist interaction with the receptor, or possibly to second messenger production, or to events beyond those in neurons on which D_1 receptors reside.

The NO donor ARG alone potently induced oral activity in 6-OHDA-lesioned rats, to a level 50% greater than was found with the DA D_1 agonist SKF 38393. However, SKF 38393 did not have an additive effect with ARG. The 5-HT_2 agonist m-chlorophenylpiperazine (Gong and Kostrzewa 1992, Brus et al. 1994) and the muscarine agonist pilocarpine (Kostrzewa and Neely 1993) each capably produce oral activity responses that are 50 to 100% greater than is found after SKF 38393.

ARG lacked an effect alone on catalepsy but enhanced SCH 23390-induced catalepsy. This finding, in conjunction with the observed induction of oral activity by ARG in 6-OHDA-lesioned rats, suggests that ARG must exert its effect at a site other than the D_1 receptor, for, a substance cannot act simultaneously as a potent agonist and antagonist. It is more likely that ARG acts in several messenger production or on the relevant neural circuitry downstream from neurons with D_1 receptors.

It may be unsettling at first that an NO donor and NO synthase inhibitor are prepared to act at different sites associated with DA D_1-mediated behavioral responses. However, NAME would tend to lower NO, and it is quite conceivable that this effect would attenuate D_1 agonist effects. In contrast, ARG generates NO. It is less likely that additioned cellular NO would add to an effect if adequate NO were already present. For these reasons NAME might be producing greater effects at sites associated with the D_1 receptor.

The major conclusion for this study is that NO is a likely candidate second messenger for DA D_1 receptors, analogous to its role for DA D_3 receptors, and that NO appears to be associated with DA receptor supersensitization.

ACKNOWLEDGEMENTS

Authors wish to thank Mrs W. Tramer and U. Mikołajun for excellent technical assistance. This study was sup-

ported by grant NN-1-30/97 from The Silesian Academy of Medicine. It was developed subsequent to a grant from the Fogarty International Center, Health Scientific Exchange Program with Poland (R.M.K.)

REFERENCES

Barone P., Davis T.A., Braun A.R., Chase T.N. (1986) Dopaminergic mechanisms and motor function: Characterization of D_1 and D_2 dopamine receptor interaction. Eur. J. Pharmacol. 123: 109-114.

Breese G.R., Duncan G.E., Napier T.C., Bondy S.C., Iorio L.C., Mueller R.A. (1987) 6-Hydroxydopamine treatments enhance behavioral response to intracerebral microinjection of D_1 and D_2 dopamine agonists into nucleus accumbens and striatum without changing dopamine antagonist binding. J. Pharmacol. Exp. Ther. 240: 167-176.

Brus R., Kostrzewa R.M., Perry K.W., Fuller R.W. (1994) Supersensitization of the oral res ponse to SKF 38393 in neonatal 6-hydroxydopamine-lesioned rats is eliminated by neonatal 5,7-dihydroxytryptamine treatments. J. Pharmacol. Exp. Ther. 268: 231-237.

Brus R., Szkilnik R., Kostrzewa R.M. (1996) Nitric oxide (NO) and central dopamine (DA) D_3 receptor reactivity to quinpirole in rats. Acta Neurobiol. Exp. 56: 15-19.

Calne D.B. (1984) Progress in Parkinsons disease. N. Engl. J. Med. 310: 523-524.

Damsma G., Bottema T., Westernik B.H.C., Tepper P.G., Dijkstra D., Pugsly T.A., MacKenzie R.G., Heffner T.G., Wikstrom H. (1993) Pharmacological aspects of R-(+)-7-OH-DPAT, a putative dopamine D_3 receptor ligand. Eur. J. Pharmacol. 249: R9-R10.

Gong L., Kostrzewa R.M. (1992) Supersensitized oral response to a serotonin agonist in neonatal 6-OHDA-treated rats. Pharmacol. Biochem. Behav. 41: 621-623.

Hamdi A., Kostrzewa R.M. (1991) Ontogenic homologous supersensitization of dopamine D_1 receptors. Eur. J. Pharmacol. 203: 115-120.

Iorio L.C., Barnett A., Billard W., Gold E.H. (1986) Benzodiazepines: Structure - activitiy relationships between D_1 receptor blockade and selected pharmacological effects. Adv. Exp. Med. Biol. 204: 1-14.

Koller W.C., Herbster L. (1988) D_1 and D_2 dopamine receptor mechanisms in dopaminergic behaviors. Clin. Neuropharmacol. 11: 221-231.

Kostrzewa R.M., Gong L. (1991) Supersensitized D_1 receptors mediate enhanced oral activity after neonatal 6-OHDA. Pharmacol. Biochem. Behav. 39: 677-682.

Kostrzewa R.M., Hamdi A. (1991) Potentation of spiperone-induced oral activity in rats after neonatal 6-hydroxydopamine. Pharmacol. Biochem. Behav. 38: 215-218.

Kostrzewa R.M., Neely D. (1993) Enhanced pilocarpine-induced oral activity responses in neonatal 6-OHDA-treated rats. Pharmacol. Biochem. Behav. 45: 737-740.

Lin A.M., Kao L.S., Chai C.Y. (1995) Involvement of nitric oxide in dopaminergic transmission in rat striatum: an in vivo electrochemical study. J. Neurochem. 65: 2043-2049.

Lonart G., Cassels K.L., Johnson K.K. (1993) Nitric-oxide induces calcium-dependent ^{3}H-dopamine release from striatal slices. J. Neurosci. Res. 35: 192-198.

Marras R.A., Martis A.P., Del Bel E.A., Gnimaraes F.S. (1995) L-NOARG, an inhibitor of nitric oxide synthase, induces catalepsy in mice. NeuroReport 7: 158-160.

Melis M.R., Argiolas A. (1993) Nitric oxide synthase inhibitors prevent apomorphine - and oxitocin - induced penile erection and yawning in male rats. Brain Res. Bull. 32: 71-74.

Pogun S., Bauman M.H., Kuhar M.J. (1994) Nitric oxide inhibits ^{3}H-dopamine uptake. Brain Res. 641: 83-91.

Rosengarten H., Schweitzer J.W., Egava J., Friedhoff A.J. (1986) Diminished dopamine receptor function and the emergence of repetitive jaw movements. Adv. Exp. Med. Biol. 235: 159-167.

Rosengarten H., Schweitzer J.W., Friedhoff A.J. (1993) Induction of oral dyskinesas in naive rats by D_1 stimulation. Life Sci. 33: 2479-2482.

Sokoloff P., Martres M.P., Schwarz J.C. (1993) La famille des recepteurs de la dopamine. Synthese 9: 12-20.

Waddington J.L. (1990) Spontaneous orofacial morements induced in rodents by very long-term neuroleptic drug administration: phenomenology, pathophysiology and putative relationship to tardive dyskinesia. Psychopharmacology (Berl.) 101: 431-447.

Zhu X.Z., Luo L.G. (1992) Effect of nitroprusside (nitric oxide) on endogenous dopamine release from rat striatal slices. J. Neurochem. 59: 932-935.

Received 15 January 1997, accepted 2 August 1997