Pharmacology Biochemistry and Behavior, Vol. 44, pp. 865–868, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Biphasic Effects of Dopamine Agonist N-0434 on Locomotor Behaviors in Rats

MERLE E. MEYER[1] AND THOMAS J. POTTER

Department of Psychology, University of Florida, Gainesville, FL 32611

Received 14 May 1992

MEYER, M. E. AND T. J. POTTER. *Biphasic effects of dopamine agonist N-0434 on locomotor behaviors in rats.* PHARMACOL BIOCHEM BEHAV 44(4) 865–868, 1993.—The effects of a dopamine agonist, (±)-2-(*N*-penylethyl-*N*-propyl)amino-5-hydroxytetralin (N-0434) (SC doses of 0.00, 0.01, 0.1, and 1.0 mg/kg) were tested in rats for 120 min in an activity monitor. The durations in seconds of horizontal locomotor time, rearing time, stereotypy time, and margin time (thigmotaxis) were measured during 12 10-min time blocks. N-0434 (0.1 and 1.0 mg/kg) resulted in biphasic effects (initial inhibition followed by potentiation) of linear locomotor time and an attenuation of thigmotaxis. The 0.1- and 1.0-mg/kg doses initially suppressed rearing time but had mixed potentiation effects. The 0.01- to 1.0-mg/kg doses suppressed stereotypy time. The differential behavioral profiles were discussed in reference to the functions of dopamine receptors.

N-0434 Dopamine D_2 receptor Locomotion Rats Rearing Stereotypy Thigmotaxis

THE existence of subtypes of dopamine (DA) receptors has become well accepted. During the last decade, the DA receptors were classified as either D_1 and D_2 receptor subtypes (17). The D_1 receptor has been typically associated with the stimulation of adenylate cyclase and the D_2 receptor is either independent of adenylate cyclase or mediates its inhibition. The availability of agonists and antagonists acting primarily at the D_1 or D_2 receptor sites has stimulated research to characterize the functional effects of each receptor subtype. It has been suggested that both the D_1 and D_2 receptor sites must be stimulated to achieve the full range of DA-induced behaviors.

LY141865 and its active enantiomer LY171555 (quinpirole), D_2 agonists, differentially influence a range of range of behaviors in a biphasic function (3,6,9,10,12,19,26). It is thought that the initial hypoactivity was a function of the autoreceptors and that the hyperactivity is a function of postsynaptic stimulation (28). While LY171555 is a potent D_2 agonist (20), LY171555 in high concentrations binds to α_2- and H_2-receptors and it is known that the α_2-agonists can cause biphasic effects (1,4,13).

In addition to the partial ergolines, a number of D_2 agonists have been developed. One is the 2-aminotetralins; the first was (±)-2-(*N*-Phenylethyl-*N*-propyl)amino-5-hydroxytetralin (N-0434) (7), followed by N-0437 (18). These compounds were inactive in stimulating D_1 receptors, were more potent than bromocriptine, LY141865 and LY171555, and did not have a high affinity for other receptor sites (2, 5,7,8,22–25). [^{3}H]N-0437 has been used to label D_2 receptors (21,23).

Recently, molecular biology has provided a new perspective on DA receptors and binding sites. In addition to the two main DA subtypes, D_1 and D_2, there are at least three more subtypes (15). The DA D_3 receptor has been identified within the limbic system and represents both an autoreceptor and a postsynaptic receptor. The D_3 receptor pharmacology resembles the D_2 receptor (16). The D_4 receptor approximates the D_2 and D_3 receptors (27), while the D_5 is more similar to the D_1 (18).

Because of the high potency of the 2-aminotetralins and the low affinity for other receptor sites, the present study investigated the effects of the selective D_2 agonist, N-0434, on various patterns of locomotor activities. As the relationship to other DA subtypes for N-0434 has not been reported, it is assumed that this DA agonist was D_2 subtype.

METHOD

Animals

Male Long-Evans rats weighing 200–230 g were obtained from Charles River. Rats were individually housed in stainless steel cages, had food and water ad lib, and were maintained on a 12 L : 12 D (light 0700–1900 h) cycle. Animals were tested in the light phase between 1000–1600 h. The room in which animals were maintained was a constant temperature (21 ± 2°C). This study was carried out in compliance with the rules set forth in the NIH Guide for the Care and Use of Laboratory Animals.

[1] To whom requests for reprints should be addressed.

Drug and Drug Administration

The DA D_2 receptor agonist N-0434 HCl (MW 345.9) was obtained from Research Biochemicals Inc. (Natick, MA). The drug was dissolved in distilled water. Distilled water was also given for the vehicle control condition (0.00 mg/kg). The drug solutions were made up daily to the appropriate concentrations of 0.01, 0.1, and 1.0 mg/kg and were injected SC.

Apparatus

Immediately after injection, animals were placed into activity chambers (Omnitech Digiscan Animal Activity Monitor) for 120 min. The acrylic cage within the monitor measured approximately 42 × 42 × 30.5 cm. The monitor was equipped with 16 beams 2.54 cm apart from front to back and from side to side. The Digiscan analyzer converted the patterns of the beams broken into different measures of locomotor activity. The measures analyzed in this study were the horizontal movement time in seconds, rearing time in seconds, stereotypy time in seconds, and margin time in seconds. Horizontal movement time was the amount of time an animal was during a given time period. As long as an animal was moving, this measure was increased in seconds. If the animal was not moving for more than 1 s, this measure was not incremented. Rearing time was the amount of time an animal reared above the vertical sensors. As long as an animal reared above these vertical sensors, this measure was increased in seconds. Stereotypy time was the amount of time an animal was repeated breaking the same sensor beam or sets of beams. This activity measure has been shown to be associated with grooming, head bobbing, and chewing (14). The margin time was the amount of time an animal spent within 1 cm to the proximity to the walls (14).

Statistics

The treatment groups of 0.01 and 0.1 mg/kg had 10 animals each, and the 0.00- and 1.0-mg/kg groups each had 8 animals; all animals were randomly assigned. A two-factor mixed-design analysis of variance (ANOVA) was used to analyze the within measures (12 10-min time blocks), between the treatment conditions (four dose levels), and the time blocks × dose treatment interaction effects for each of the four measures of locomotor activity. The significant interactions for the dose × time intervals were followed up with Dunnett's multiple-comparison tests between the vehicle control group (0.00 mg/kg) and the treatment groups at each of the 12 10-min time blocks. Because of the biphasic effects, the main effects of the difference between treatment groups and the differences across time blocks are be reported; however, no subsequent analyses of those differences were made. p values equal to or less than 0.05 were judged to be statistically significant.

RESULTS

Horizontal Movement Time

Figure 1A illustrates the biphasic effects of N-0434 on horizontal movement time in seconds as a function of four dose levels (0.00, 0.01, 0.1, and 1.0 mg/kg) over the 12 10-min time course. The overall ANOVA revealed a significant difference among the four dose levels, $F(3, 32) = 13.78$, $p < 0.001$; a significant difference across the 12 10-min time blocks, $F(11, 352) = 2.15$, $p = 0.016$; and a significant doses × time intervals interaction, $F(33, 352) = 3.99$, $p < 0.001$. Subsequent analyses between the 1.0-mg/kg group and the vehicle control group (0.00 mg/kg) disclosed a biphasic effect. Initially, at the 10-min time block there was a significant hypoactivity ($p < 0.05$), and at the 20- to 120-min time blocks there was hyperactivity ($ps < 0.01$). The profile for the 0.1-mg/kg groups showed at 10 min significant hypoactivity ($p < 0.05$)

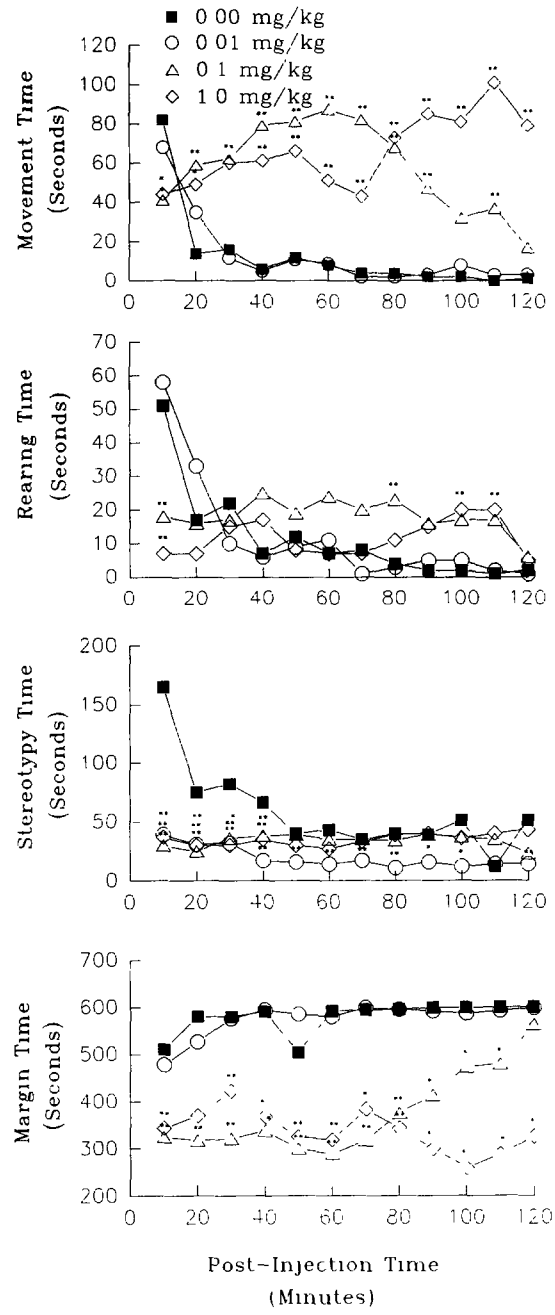

FIG. 1. Effects of several doses of the dopamine D_2 agonist N-0434 on four measures of locomotor activity in seconds over 120 min. (A), horizontal movement time; (B), rearing time; (C), stereotypy time; (D), margin time. Significant differences from the vehicle control group (0.00 mg/kg) at each time point: $*p < 0.05$, $**p < 0.01$. For clarity, the error bars have not been included.

and significant hyperactivity from 20–90 min ($ps < 0.01$) and at the 110-min block ($p < 0.05$). At the end of the 120-min block, the difference between the 0.1- and 0.00-mg/kg groups was not significant ($p > 0.05$). There were no significant differences between the 0.01- and the 0.00-mg/kg groups at any time block ($ps > 0.05$).

Rearing Time

The rearing time in seconds of rats treated with one of the four dose levels of N-0434 (0.00, 0.01, 0.1, and 1.0 mg/kg) over the 12 10-min time courses is shown in Fig. 1B. The ANOVA indicated a nonsignificant dose effect ($p > 0.05$); a significant time course, $F(11, 352) = 6.14$, $p < 0.001$; and a significant doses × time intervals interaction, $F(33, 352) = 3.05$, $p < 0.001$. At the 10-min time block, both the 0.1- and 1.0-mg/kg groups showed significant suppression of rearing time when compared to the vehicle controls ($ps < 0.01$). Only at time block 80 min for the 0.1-mg/kg group and at time blocks 100 and 110 min for the 1.0-mg/kg group was any potentiation of rearing shown ($p < 0.05$). All other comparisons were not significant ($p > 0.05$).

Stereotypy Time

Stereotypy time in seconds is portrayed in Fig. 1C. The ANOVA resulted in a significant dose effect, $F(3, 32) = 24.21$, $p < 0.001$; a significant time course effect, $F(11, 352) = 10.99$, $p < 0.001$; and a significant dose × time course interaction, $F(33, 352) = 7.96$, $p < 0.001$. The subsequent analyses shown that N-0434 attenuated stereotypy activity during the first 40 min ($ps < 0.01$) and that, for the most part, the smallest dose (0.01 mg/kg) continued the attenuation effect over the 120 min. On the other hand, the larger dose levels did not potentiate stereotypy behaviors across the 120-min time course.

Margin Time

Figure 1D illustrates the margin time in seconds or thigmotaxis of rats. The ANOVA indicated a significant N-0434 dose effect, $F(3, 32) = 38.58$, $p < 0.001$; a significant time course, $F(11, 352) = 2.71$, $p = 0.002$; and a significant dose × time course interaction. Both the 0.00- and 0.01-mg/kg groups show typical thigmotaxis. On the other hand, the 0.1- and 1.0-mg/kg groups significantly differ from controls through the 90-min time blocks ($ps < 0.01$); at the time blocks of 100 and 110 min, the 0.1-mg/kg group differed from controls ($ps < 0.05$). However, by the end of the 120 min the 0.1-mg/kg group did not differ from controls ($p > 0.05$), whereas the 1.0-mg/kg group was significantly different from controls at every time block ($p < 0.01$) and thus showed an attenuation of thigmotaxis.

DISCUSSION

Typically, it has been argued that low dosages of DA agonists attenuate locomotor activities and that high dosages potentiate locomotor activities. It was thought that the attenuation is due to the inhibitory effects of the autoreceptors and that the potentiation is caused by excitation effects of the postsynaptic receptor stimulation (28). The biochemical evidence for N-0437 supports this argument as small doses inhibit the release of DA through the stimulation of the D_2 autoreceptors and higher doses are agonists at the postsynaptic D_2 receptors (22,24,25).

The present data would not support the literal hypotheses as stated. N-0434 in a dose × time course produced biphasic changes in horizontal locomotor time. The dosage of 1.0 mg/kg resulted in an initial significant hypoactivity, followed by a continuous increase in hyperactivity over the 120-min time course. In addition, the 0.1-mg/kg dosage also resulted in an initial hypoactivity, followed by an increase in activity that reached an asymptote at 60 min, and then followed by a continuous decrease in horizontal movement. The lowest dose level (0.01 mg/kg) mirrored the vehicle control group. Somewhat comparable results were seen in thigmotaxis. Both the control or 0.00- and 0.01-mg/kg groups emitted thigmotaxis through the 120-min session, whereas the 1.0-mg/kg group emitted significantly less across the time course. The 0.1-mg/kg group showed intermediate effects.

The effects of N-0434 on rearing and stereotypy times were mixed and do not fully support the current hypotheses. The data clearly showed that the 0.1- and 1.0-mg/kg dosages initially inhibited rearing. However, the activation phase effects were minimal except for three data points late in the session. Again, the lowest dosage had no effect. On the other hand, all three dose levels of N-0434 (0.01, 0.1, and 1.0 mg/kg) suppressed stereotypy time through the first 40 min and the lowest dose (0.01 mg/kg) continued to suppress stereotypy at most time points. From an observational analysis, these three dose levels inhibited the low-intensity behaviors of stereotypy, such as grooming and sniffing, and also diuresis (Meyer and Potter, unpublished data).

In accordance with these analyses, caution should be exercised from assuming that the locomotor stimulation and increased stereotypy behaviors induced in the intact rat by apomorphine generalizes to the D_2 agonists. The enigma of the biphasic effects of the D_2 agonists may be resolved, at least for N-0437, once the (+) and (−) enantiomers become available (25) and once the pharmacology for the D_3 and D_4 receptors is ascertained (15).

ACKNOWLEDGEMENTS

The authors thank Bonnie McLaurin and Jeanene Griffin for their excellence assistance. This research was supported by a Whitehall Foundation grant to M.E.M.

REFERENCES

1. Armstrong, J. M.; Duval, N.; Langer, S. Z. LY141865 stimulates histamine-2(H-2) receptors and dopamine-2 (DA-2) receptors in the anaesthetized dog. Eur. J. Pharmacol. 87:165–166; 1983.
2. Beaulieu, M.; Itoh, Y.; Tepper, P.; Horn, A. S.; Kebabian, J. W. *N,N*-Disubstituted 2-aminotetralins are potent D-2 dopamine receptor agonists. Eur. J. Pharmacol. 105:15–21; 1984.
3. Clifton, P. G.; Rusk, I. N.; Cooper, S. J. Stimulation and inhibition of food intake by the selective dopamine D_2 agonist, N-0437: A meal pattern analysis. Pharmacol. Biochem. Behav. 33:21–26; 1989.
4. Cohen, M. L.; Shaar, C. J.; Colbert, W. E. α_2-Agonist activity of the dopamine DA2 agonists LY171555. J. Cardiovasc. Pharmacol. 6:1245–1248; 1984.
5. Dubocovich, M. L.; Hensler, J. G. Functional presynaptic D-2 and postsynaptic D-1 dopamine receptors in the central nervous system. In: Beart, P. M.; Woodruff, G. N.; Jackson, D. M., eds. Pharmacology and functional regulation of dopaminergic neurons. Littleton, MA: PSG Publishing; 1988:175–177.
6. Eilam, D.; Szechtman, H. Biphasic effect of D-2 agonist quinpir-

ole on locomotion and movements. Eur. J. Pharmacol. 161:151–157; 1989.
7. Horn, A. S.; Tepper, P.; Kebabian, J. W.; Beart, P. M. N-0434, a very potent and specific new D-2 dopamine receptor agonist. Eur. J. Pharmacol. 99:125–126; 1984.
8. Horn, A. S.; van der Weide, J.; Tepper, P. G.; de Vries, J. B.; Bosker, F. J.; Dijk, J.; Moller, W. The development of potent and selective D-2 agonist of the 2-aminotetralin group: Their clinical applications and their use in the isolation of the D-2 receptor. In Beart, P. M.; Woodruff, G. N.; Jackson, D. M., eds. Pharmacology and functional regulation of dopaminergic neurons. Littleton, MA: PSG Publishing; 1988:16–21.
9. Meyer, M. E.; Meyer, M. E.; Potter, T. J.; Van Hartesveldt, C. Differential effects of dopamine D_2 agonist quinpirole upon the dorsal immobility response in rats. Pharmacol. Biochem. Behav. 42:257–259; 1992.
10. Monti, J. M.; Jantos, H.; Fernandez, M. Effects of the selective dopamine D-2 receptor agonist, quinpirole on sleep and wakefulness in the rat. Eur. J. Pharmacol. 169:61–66; 1989.
11. Murray, A. M.; Waddington, J. L. The induction of grooming and vacuous chewing by a series of selective D-1 dopamine receptor agonists: Two directions for D-1:D-2 interaction. Eur. J. Pharmacol. 160:377–384; 1989.
12. Rubinstein, M.; Gershanik, O; Stefano, F. J. E. Different roles of D-1 and D-2 dopamine receptors involved in locomotor activity of supersensitive mice. Eur. J. Pharmacol. 148:419–426; 1988.
13. Ruffolo, R. R.; Shaar, C. J. Relative potency of LY141865 at dopamine-DA2 and histamine-H_2 receptors. Eur. J.Pharmacol. 92:295–296; 1983.
14. Sanberg, P. R.; Zoloty, S. A.; Willis, R.; Ticarich, C. D.; Rhoads, K.; Nagy, R. P.; Mitchell, S. G.; Laforest, A. R.; Jenks, J. A.; Harkabus, L. J.; Gurson, D. B.; Finnefrock, J.A.; Bednarik, E. J. Digiscan activity: Automated measurement of thigmotactic and stereotypic behavior in rats. Pharmacol. Biochem. Behav. 27:569; 1987.
15. Sibley, D. R.; Monsma, F. J., Jr. Molecular biology of dopamine receptors. Trends Pharmacol. Sci. 13:61–69; 1992.
16. Sokoloff, P.; Giros, B.; Martres, M.-P.; Bouthenet, M.-L.; Schwartz, J.-C. Molecular cloning and characterization of a novel dopamine receptor (D_3) as a target for neuroleptics. Nature 347: 146–151; 1990.
17. Stoof, J. C.; Kebabian, J. W. Two dopamine receptors: Biochemistry, physiology and pharmacology. Life Sci. 53:2281–2296; 1984.
18. Sunahara, R. K.; Guan H.-C.; O'Dowd, B. F.; Seeman, P.; Laurier, L. G.; Ng, G.; George, S. R.; Torchia, J.; Van Tol, H. H. M.; Niznik, H. B. Cloning of the gene for a human dopamine D_5 receptor with higher affinity for dopamine than D_1. Nature 350: 614–619; 1991.
19. Timmerman, W.; Rusk, I. N.; Tepper, P.; Horn, A. S.; Cooper, S. J. The effects of the enantiomer of the dopamine agonist N-0437 on food consumption and yawning behaviour in rats. Eur. J. Pharmacol. 174:107–114; 1989.
20. Tsuruta, K.; Frey, F. A.; Grewe, C. W.; Cote, T. E.;Eskay, R. L.; Kebabian, J.W. Evidence that LY141865 specifically stimulates the D-2 receptor. Nature 323:464–465; 1981.
21. van der Weide, J.; Camps, M.; Horn, A. S.; Palacios, J. M. Autoradiographic localization of dopamine D_2 receptors in the rat brain using the new agonist [^{3}H]N-0437. Neurosci. Lett. 83: 259–263; 1987.
22. van der Weide, J.; de Vries, J. B.; Tepper, P. G.; Horn, A. S. Pharmacological profiles of three new, potent and selective dopamine receptor agonists: N-0434, N-0437 and N-0743. Eur. J. Pharmacol. 125;273–282; 1986.
23. van der Weide, J.; de Vries, J.; Tepper, P. G.; Horn, A. S. In vitro binding of the very potent D-2 dopamine receptor agonist [^{3}H]N-0437 to calf caudate membranes. Eur. J. Pharmacol. 134: 211–219; 1987.
24. van der Weide, J.; de Vries, J. B.; Tepper, P. G.; Krause, D. N.; Dubocovich, M. L.; Horn, A. S. N-0437: A selective D_2 dopamine receptor agonist in vitro and in vivo models. Eur. J. Pharmacol. 147:249–258; 1988.
25. van der Weide, J.; Tendijck, M. E. C.; Tepper, P. G.; de Vries, J. B.; Dubocovitch, M. L.; Horn, A. S. The enantiomers of the D_2 dopamine receptor agonist N-0437 discriminate between pre and postsynaptic dopamine receptors. Eur. J. Pharmacol. 146: 319–326; 1988.
26. Van Hartesveldt, C.; Cottrell, G. A.; Potter, T.; Meyer, M. E. Effects of intracerebral quinpirole on locomotion in rats. Eur. J. Pharmacol. 214:27–32; 1992.
27. Van Tol, H. H. M.; Bunzow, J. R.; Guan, H.-C.; Sunahara, R. K.; Seeman, P.; Niznik, H. B.; Civelli, O. Cloning of the gene for a human dopamine D_4 receptor with high affinity for the antipsychotic clozapine. Nature 350:610–614; 1991.
28. Wolf, M. E.; Roth, R.H. Dopamine autoreceptors. In: Creese, I.; Fraser, C. M., eds. Dopamine receptors. New York: Alan R. Liss; 1987:45–96.