Pharmacology Biochemistry and Behavior, Vol. 44, pp. 157–160, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Intracerebral Haloperidol Potentiates the Dorsal Immobility Response in the Rat

MERLE E. MEYER,[1] GEORGIA A. COTTRELL AND CAROL VAN HARTESVELDT

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

Received 20 April 1992

MEYER, M. E., G. A. COTTRELL, AND C. VAN HARTESVELDT. *Intracerebral haloperidol potentiates the dorsal immobility response in the rat.* PHARMACOL BIOCHEM BEHAV 44(1) 157–160, 1993.—The effects of intracerebral microinjections of 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone (haloperidol) (1.0 μg, 0.5 μl) in five regions of the brain were tested on the duration of the dorsal immobility response (DIR) and the cling and bar catalepsy in the rat. The duration of the DIR was significantly potentiated (but not the cling and bar catalepsy) following 2-h postinjection of haloperidol in the caudate putamen, nucleus accumbens, and globus pallidus but not in the substantia nigra pars compacta or cortex. These data further expand the previous evidence of regional variations in dopamine to the effects upon inhibitory behaviors.

Haloperidol Dorsal immobility response Caudate putamen Cortex Nucleus accumbens Globus pallidus Substantia nigra pars compacta

THE existence of D_1 and D_2 subtypes of dopamine (DA) receptors is in general accepted (13,25,27), and selective agonists and antagonists at these receptor sites have been developed. The D_1 receptor activates adenylate cyclase and [^{3}H]SCH 23390 binds to this subtype (2). The D_2 receptor subtype inhibits or has no effect on adenylate cyclase and binds to [^{3}H]4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone (haloperidol) (26). However, more than two forms of the D_2 receptor have been suggested and a possible D_3 receptor has been isolated (12,28). Haloperidol has a 30-fold greater affinity for the DA D_2 receptor than for the D_1 receptor (1) and 10- to 20-fold preference for D_2 over D_3 (28).

For the butyrophenone ligands, the D_2 receptor binding is found in the majority of tissues innervated with DA. However, the DA D_2 receptors are present in several DA-rich regions of the brain that are innervated by two or more primary DA neuronal pathways. The mesostriatal system originates primarily in the substantia nigra pars compacta and secondarily in the ventral tegmental area, with their relatively low D_2 receptor density, and projects to the D_2-rich caudate putamen and Islands of Calleja and the low D_2 density globus pallidus and subthalamic nucleus. The mesolimbic DA system arises primarily in the ventral tegmental and secondarily in the substantia nigra pars compacta and projects to the D_2-rich nucleus accumbens and olfactory tubercle (22,26).

The effects of haloperidol on catalepsy have been thought to be mediated by the blockade of DA D_2 receptors located on neurons originating in the dorsal striatum (9,12,16). The more recent behavioral evidence does not warrant these conclusions. A number of studies have shown that catalepsy can be elicited by various DA D_1 antagonists (7,14,18,21), as well as by various DA D_2 antagonists (7,9,14,19).

There are regional zones within the mesostriatal (dorsal caudate putamen), mesolimbic (ventral caudate putamen, nucleus accumbens, and olfactory tubercle), and mesocortical regions that support different DA-induced responses.

The behavioral assays of the DA effects include the rotation, stereotypy and atypical chewing and myoclonic jerking behaviors, catalepsy, consummatory, locomotor activity, learning and condition, and drug discrimination [(4,5,15, 17,20); also see (6,8,29) for general reviews]. The majority of the research is associated with the various active behavioral strategies. However, behavioral inhibition that results in immobility is phylogenetically an old adaptive behavioral pattern in vertebrates and is much less investigated.

The dorsal immobility response (DIR) is a species-typical response experimentally elicited by grasping an animal by the dorsal skin at the nape of the neck and lifting the animal off its feet. In the rat, the animal immediately exhibits a stereotypical immobility posture that persists for a period of time until the animal suddenly emits escape-like behaviors (18,19,30,31). Within the context of naturally occurring inhibitory behaviors, the DIR may mimic the transport response in the young of some mammalian species when the adult picks up and carries the young by the dorsal nape (3,19). The DIR may also mimic the immobility of a prey when carried by a predator (10,11,24).

In this experiment, we focused on the anatomic substrates

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

that may underlie the inhibitory effects induced by the selective D_2 antagonist haloperidol. Microinjections of haloperidol were made in five brain regions associated with various DA systems to determine the inhibitory effects as measured by DIR and bar and cling catalepsy.

METHOD

Animals

Fifty Long-Evans hooded female rats weighing 200–225 g were obtained from Charles River. They were individually housed, had food and water ad lib, and were maintained on a 12 L : 12 D (light 0700–1900 h) cycle. This study was carried out in compliance with the rules set forth in the NIH *Guide for the Care and Use of Laboratory Animals.*

Surgery

All rats were ovariectomized (OVX) bilaterally under ether anesthesia 2 weeks prior to cannulation. Stereotaxic surgery was carried out under equithesin anesthesia. Stainless steel guide cannulae were bilaterally implanted using the following coordinates from Paxinos and Watson (23) with reference to bregma, midline, and skull surface, respectively: caudate putamen, +0.2, ±2.5, −2.5 (injection cannula, −7.0); globus pallidus, −0.8, ±2.5, −2.5 (injection cannula, −6.5); nucleus accumbens, +1.7, ±1.25, −2.5 (injection cannula, −7.0); cerebral cortex, +0.2, ±2.5, −1.0 (injection cannula −1.5); substantia nigra pars compacta, −4.3, ±2.2, −2.5 (injection cannula, −7.5). Stainless steel stylets kept the guide cannulae patent when the injection cannulae were not inserted. Animals were allowed 2 weeks recovery before behavioral testing.

Drugs

Haloperidol (Research Biochemicals Inc., Natick, MA) was dissolved in distilled water and 1% lactic acid. The vehicle control solutions were adjusted for equivalent pH. Haloperidol and the vehicle control were bilaterally injected into the various sites with a volume of 0.5 μl/side and the haloperidol dosage was 1.0 μg/side. Two hours following postinjection, the animal was behaviorally tested.

Response Measurements

Horizontal bar grasp response. A 30-cm long, 0.5-cm diameter metal bar was 8 cm high, horizontal to a table. The animal was held by its back and shoulders and moved forward to the bar with the hind legs contacting the table top. The measurement was the duration from releasing of the hand support, once the animal grasped the bar, until the animal placed both forepaws on the table or until 120 s had elapsed.

Vertical cling response. A 90° vertical grid was standard hardware cloth (10 wires/8 cm). The animal was placed on the screen in a head-up position; when it grasped the wire grid, the animal become self-supporting. The response was measured from the time of the hand release until the animal moved a paw to a different position or until 120 s had elapsed.

DIR. To induce the DIR, the animal was gently grasped by the dorsal skin at the nape of the neck and lifted off its feet with no other part of the animal's body touching any other surfaces. As all rats elicited the species-typical immobility response when the DIR was first induced, the duration was measured from the onset of the DIR until the animal emitted directed movements associated with escape-like behaviors or until 300 s had elapsed. The animal received three DIR trials during the test session with an intertrial interval of 30 s. For each animal, the durations for the three trials were averaged for a single value. The order of the three testing procedures were presented randomly, with 1 min between procedures.

Histology

After the behavioral testing was completed, the animal was administered an overdose of sodium pentobarbital (Butler, Chicago, IL) and perfused intracardially with 0.9% saline followed by 10% formalin. Brains were removed and placed in a 20% sucrose–10% formalin solution. The brains were frozen, sectioned, mounted on slides, and stained with cresyl violet, and the locations of the cannulae tips were verified. Only those animals with bilateral injection lesions in the target areas were used for the data analysis.

Statistics

A factorial design analysis of variance (ANOVA) with two factors was used to examine the effects of haloperidol–vehicle treatment conditions by five neural locations upon the durations of the three immobility measures. The independent *t*-test was used for posthoc subsequent analyses. The p values less than or equal to 0.05 were judged statistically significant.

RESULTS

Horizontal Bar and Vertical Cling Responses

There were no statistically significant differences for the horizontal bar and vertical cling behaviors with the dose level used in this study ($p > 0.05$).

DIR

The 2 × 5 factorial design ANOVA resulted in a significant drug effect, $F(1, 37) = 94.25$, $p < 0.001$, a significant site effect, $F(4, 37) = 25.51$, $p < 0.001$, and a significant drug × site effect, $F(4, 37) = 16.25$, $p < 0.001$ (see Fig. 1). The subsequent *t*-tests between the haloperidol and vehicle controls revealed that haloperidol significantly potentiated the durations of the DIR at the caudate putamen, $t(8) = 7.46$, $p < 0.001$, at the globus pallidus, $t(8) = 3.88$, $p < 0.005$, and at the nucleus accumbens, $t(7) = 8.49$, $p < 0.001$. There were no significant differences at the cortex and substantia nigra pars compacta ($p > 0.05$). There were no significant differences among the five vehicle control groups ($p > 0.05$). On the other hand, there were significant differences among the five haloperidol groups, where there were differences between the nucleus accumbens and globus pallidus, $t(7) = 2.39$, $p < 0.05$, and the caudate putamen, globus pallidus, and nucleus accumbens sites differed from the cortex and substantia nigra pars compacta sites ($t < 0.001$).

DISCUSSION

The results of the present experiment have shown that microinjections of haloperidol in the caudate putamen, globus pallidus, and nucleus accumbens, but not in the cortex or substantia nigra pars compacta, significantly potentiated the duration of the DIR in comparison with their vehicle controls. The nonsignificant effects associated with the bar and vertical cling procedures in part replicate the data reported by Costell, Naylor, and Olley (9). The dose level of 1 μg/side was too small to elicit bar or cling catalepsy.

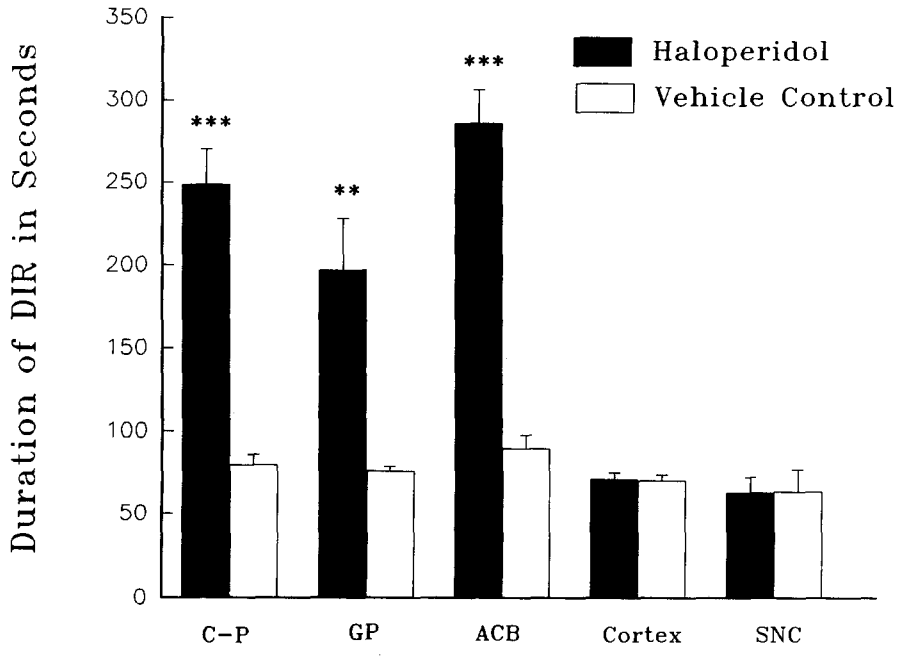

FIG. 1. Durations of the dorsal immobility response (DIR) were potentiated by bilateral microinjections of haloperidol (1.0 μg/side) in the caudate putamen (C-P) (***$p < 0.001$), nucleus accumbens (ACB) (***$p < 0.001$), and globus pallidus (GP) (**$p < 0.005$) but not in the cortex or substantia nigra pars compacta (SNC). The error bars represent ±1 SE. Asterisks represent significant differences between the haloperidol and vehicle controls within a brain region.

It has been suggested that the effects of haloperidol catalepsy are mediated by the blocking of D_2 receptors (8,12, 16,29). Our present data would clearly support such a suggestion; however, D_1 antagonists (SCH 23390 and SK&F83566) also mediate bar and cling catalepsy and potentiate the duration of the DIR (18). Further, it has been suggested that the neurons where the blockage takes place originate in the dorsal striatum (9,16). The significant potentiation of the DIR in the caudate putamen and globus pallidus and the nonsignificant effect of haloperidol within the cortex and substantia nigra pars compacta would support such a hypothesis. On the other hand, the highly significant potentiation of the DIR in the nucleus accumbens suggest an expansion of the functional D_2 sites. The nucleus accumbens receives neural input from the anterior cingulate, various cortices, the amygdala, and the hippocampus. These limbic structures have been suggested to have functional significance associated with motivation and emotion. While we did not quantify the amount of defecation during DIR, the notes suggest that there were marked differences between haloperidol and vehicle controls, in particular, with the nucleus accumbens site. The DIR effects of the DA D_2 antagonist haloperidol can now be better understood within the anatomic context within various brain regions and systems involving DA.

ACKNOWLEDGEMENTS

This research was supported by a grant to M.E.M. from the Whitehall Foundation. The authors thank Bonnie McLaurin for technical assistance and Jeanene Griffin for secretarial assistance.

REFERENCES

1. Anderson, P. H.; Gingrich, J. A.; Bates, M. D.; Dearry, A.; Falardeau, P.; Senogles, S. E.; Caron, M. G. Dopamine receptor subtypes: Beyond the D_1/D_2 classification. Trends Pharmacol. Sci. 11:231-236; 1990.
2. Billard, W.; Ruperto, V.; Crosby, G.; Iorio, L. C.; Barnett, A. Characterization of the binding of 3H- SCH 23390, a selective D-1 receptor antagonist ligand, in rat striatum. Life Sci. 35:1885-1893; 1984.
3. Brewster, J.; Leon, M. Facilitation of maternal transport by Norway rat pups. J. Comp. Physiol. Psychol. 94:80-88; 1980.
4. Bury, D.; Schmidt, W. J. Effects of systemically and intrastriatally injected haloperidol and apomorphine on grooming, feeding and locomotion in the rat. Behav. Proc. 15:269-283; 1987.
5. Carey, R. J.; Kenney, S. A delayed onset of haloperidol effects on learned escape and avoidance behavior. Pharmacol. Biochem. Behav. 28:203-208; 1987.
6. Chiodo, L. A. Dopamine-containing neurons in the mammalian central nervous system: Electrophysiology and pharmacology. Neurosci. Biobehav. Rev. 12:49-91; 1988.
7. Christensen, A. V.; Arnt, J.; Hyttel, J.; Larsen, J.; Svendsen, O. Pharmacological effects of a specific dopamine D-1 antagonists SCH 23390 in comparison with neuroleptics. Life Sci. 34:1529-1540; 1984.
8. Clark, D.; White, F. J. Review: D1 dopamine receptor—the search for a function: A critical evaluation of the D1/D2 dopamine receptor classification and its functional implications. Synapse 1:347-388; 1987.
9. Costall, B.; Naylor, R. J.; Olley, J. E. Catalepsy and circling behaviour after intracerebral injection of neuroleptic, cholinergic

and anticholinergic agents into the caudate putamen, globus pallidus and substantia nigra of the rat brain. Neuropharmacology 11:645–663; 1972.
10. Eisenberg, J. F.; Leyhausen, P. The phytogenesis of predatory behavior in mammals. Z. Tierpsychol. 30:59–93; 1972.
11. Gallup, G. G., Jr. Tonic immobility: The role of fear and predation. Psychol. Rec. 27:41–61; 1977.
12. Joyce, J. N. Multiple dopamine receptors and behavior. Neurosci. Biobehav. Rev. 7:227–256; 1983.
13. Kebabian, J. W.; Calne, D. B. Multiple receptors for dopamine. Nature 277:93–96; 1979.
14. Klemm, W. R.; Block, H. D-1 and D-2 receptor blockage have additive cataleptic effects in mice, but receptor effects may interact in opposite ways. Pharmacol. Biochem. Behav. 29:223–229; 1988.
15. Kovacs, G. L.; deWied, D. Effects of amphetamine and haloperidol on avoidance behavior and exploratory activity. Eur. J. Pharmacol. 53:103–107; 1978.
16. Lowery, S. P.; Barkley, P.; Baez, L. A. The influence of anterior neostriatal lesions on the behavioral effects of spiroperidol in rats. Prog. Neuropsychopharmacol. 4:269–276; 1980.
17. Lynch, M. R.; Carey, R. J. Chronic low-dose haloperidol effects on self-stimulation rate-intensity functions. Psychopharmacology (Berl.) 102:122–129; 1990.
18. Meyer, M. E.; Cottrell, G. A.; Van Hartesveldt, C. Dopamine D1 antagonists potentiate the durations of bar and cling catalepsy and the dorsal immobility response in the rat. Pharmacol. Biochem. Behav. 41:507–510; 1992.
19. Meyer, M. E.; Smith, R. L.; Van Hartesveldt, C. Haloperidol differentially potentiates tonic immobility, the dorsal immobility, and catalepsy in the developing rat. Dev. Psychobiol. 17:383–389; 1984.
20. Miller, R.; Wickens, J. R.; Beninger, R. J. Dopamine D-1 and D-2 receptors in relation to reward and performance: A case for the D-1 receptor as a primary site of therapeutic action of neuroleptic drugs. Prog. Neurobiol. 34:143–183; 1990.
21. Morelli, M.; DiChiara, G. Catalepsy induced by SCH 23390 in rats. Eur. J. Pharmacol. 117:179–185; 1985.
22. Muller, P.; Seeman, P. Brain neurotransmitter receptors after long term haloperidol: Dopamine, acetylcholine, serotonin, α-noradrenergic and naloxone receptors. Life Sci. 21:1751–1758; 1977.
23. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1986.
24. Sargeant, A. B.; Eberhardt, L. E. Death feigning by duck in response to predation by red foxes (*Vulpes fulva*). Am. Midl. Nat. 94:108–119; 1975.
25. Seeman, P. Brain dopamine receptors. Pharmacol. Rev. 32:229–313; 1980.
26. Seeman, P.; Chau-Wong, M.; Tedesco, J.; Wong, K. Brain receptors for antipsychotic drugs and dopamine: Direct binding assays. Proc. Natl. Acad. Sci. USA 72:4376–4380; 1975.
27. Seeman, P.; Grigoriadis, D. Dopamine receptors in brain and periphery. Neurochem. Int. 10:1–25; 1987.
28. Strange, P. G. Interesting times for dopamine receptors. Trends Neurosci. 14:43–45; 1991.
29. Waddington, J. L.; O'Boyle, K. M. Drugs acting on brain dopamine receptors: A conceptual re-evaluation five years after the first selective D-1 antagonists. Pharmacol. Ther. 43:1–52; 1989.
30. Webster, D. G.; Lanthorn, T. H.; Dewsbury, D. A.; Meyer, M. E. Tonic immobility and dorsal immobility in twelve species of muroid rodents. Behav. Neural Biol. 31:32–41; 1981.
31. Webster, D. G.; Lanthorn, T. H.; Meyer, M. E. Immobility responses in *Anolis carolinensis*. Physiol. Psychol. 7:451–453; 1979.