Pharmacology Biochemistry & Behavior, Vol. 13, pp. 633–636. Printed in the U.S.A.

Inhibition of Neuroleptic-Induced Dopamine Receptor Supersensitivity by Cyclo (Leu-Gly)

HEMENDRA N. BHARGAVA AND RONALD F. RITZMANN

Department of Pharmacognosy and Pharmacology and Department of Physiology and Biophysics
University of Illinois at the Medical Center, Chicago, IL 60612

Received 12 June 1980

BHARGAVA, H. N. AND R. F. RITZMANN. *Inhibition of neuroleptic-induced dopamine receptor supersensitivity by cyclo (Leu-Gly).* PHARMAC. BIOCHEM. BEHAV. 13(5) 633–636, 1980.—Behavioral supersensitivity of dopamine receptors was induced in mice by chronic administration of haloperidol (1 mg/kg/day for 21 days) and its subsequent withdrawal for 48 hr. This was evidenced by enhanced spontaneous locomotor activity and hypothermic responses to a dopamine agonist, apomorphine. Concurrent administration of cyclo (Leu-Gly), the enzymatically resistant diketopiperazine, an analog of melantropin release inhibiting factor, blocked haloperidol-induced dopamine receptor supersensitivity as evidenced by the blockade of apomorphine induced responses. Since many studies have linked the development of neuroleptic induced tardive dyskinesias with enhanced sensitivity of brain dopamine receptors, and the latter was blocked by cyclo (Leu-Gly), this agent may be of value in preventing the development of symptoms of neuroleptic-induced tardive dyskinesias.

Cyclo (Leu-Gly) Haloperidol Apomorphine Dopamine receptor Supersensitivity Neuroleptic
Tardive dyskinesias Mice Hypothermia Locomotor activity

CONSIDERABLE evidence suggests that chronic treatment with neuroleptic drugs produces supersensitivity of brain dopaminergic systems. This supersensitivity is manifested in exaggerated response to dopaminergic agonists administered after neuroleptic treatment is terminated. For instance, dopamine agonists elicit more intense stereotyped chewing, gnawing, and aggression following long term treatment with neuroleptic drugs [18, 19, 24, 32, 33]. Long term administration of neuroleptic drugs results in the development of a neurological syndrome known as tardive dyskinesias, a condition in which abnormal involuntary oral-facial movements (dyskinesias) appear [11]. The symptoms are difficult to treat and may persist for months or years after discontinuing the drug [10].

It has been suggested that tardive dyskinesia is a result of postsynaptic dopaminergic receptor supersensitivity [23] induced by protracted pharmacological blockade of dopaminergic neurotransmission by the neuroleptics [12,35]. Biochemical evidence has also been presented for the altered brain dopaminergic sensitivity following long term treatment with neuroleptics [26]. These studies indicated that in rats, chronic haloperidol administration and subsequent withdrawal significantly increased the specific binding of tritiated haloperidol and tritiated apomorphine in the striatum and mesolimbic regions of the brain. The binding to serotonergic and alpha-adrenergic receptors was either not altered or produced a minimal change. Thus selective increments in dopamine or neuroleptic receptors (receptor proliferation) may form the basis of dopaminergic supersensitivity. Similar biochemical evidence of enhanced dopamine receptor sensitivity has been obtained in rats treated chronically with phenothiazine neuroleptics [5, 6, 32]. The supersensitivity of dopamine receptors has been proposed as an animal model of tardive dyskinesia which develops in humans after long term administration of neuroleptic drugs [23,24].

In addition to the neuroleptics, chronic administration of narcotics also leads to the development of supersensitivity of postsynaptic dopamine receptors, as a result of chronically decreased dopaminergic neurotransmission [27]. This was evidenced by an increased response to a direct acting dopamine receptors agonist, apomorphine. The dopamine receptor supersensitivity has also been confirmed by the measurement of striatal dopamine turnover [20] as well as the increase in dopamine stimulated adenylate cyclase activity in the caudate nuclei [21] of the chronically morphine treated rats. We have shown previously that chronic administration of opiates, which results in the development of tolerance and physical dependence, is associated with the development of dopamine receptor supersensitivity in mice [30] and rats [3]. This supersensitivity development, as well as the development of tolerance and dependent states are blocked by cyclo (Leu-Gly) [3,30]. It was therefore of interest to determine whether the neuroleptic induced dopamine receptor supersensitivity is affected by cyclo (Leu-Gly).

In the present communication, the effects of concurrent administration of cyclo (Leu-Gly) on the development of behavioral supersensitivity of dopamine receptors induced by long term administration of haloperidol is described.

METHOD

Male Swiss Webster mice (Scientific Small Animal Co., Arlington Heights, IL) weighing 20–25 g were used. The animals were housed in rooms with controlled temperature

Copyright © 1980 ANKHO International Inc.—0091-3057/80/110633-04$00.90/0

TABLE 1

EFFECT OF CYCLO (LEU-GLY) ON HALOPERIDOL INDUCED DOPAMINE RECEPTOR SUPERSENSITIVITY IN MICE

Treatment*	N	Spontaneous motor activity following apomorphine injection. Counts/15 min Mean ± SEM.	Change in rectal temperature at 30 min after apomorphine injection, °C.† Mean ± SEM.
Water + vehicle§	8	263 ± 51	−1.23 ± 0.22
Cyclo (Leu)Gly) + vehicle	8	320 ± 42	−1.38 ± 0.32
Water + haloperidol	20	492 ± 32‡	−2.11 ± 0.11‡
Cyclo (Leu-Gly) + haloperidol	20	280 ± 31	−1.07 ± 0.13

*Refers to daily treatment for 21 days. Responses to apomorphine (1 mg/kg) were determined 48 hr after the last injection. N represents the number of mice used.
†Difference between prior to and at 30 min after apomorphine (1 mg/kg) injection.
‡$p<0.05$ vs all other groups.
§0.01 M tartaric acid.

(23±1°C), humidity (65±2%) and light (L0600–1800 hr). Food and water were available at all times.

Cyclo (Leu-Gly) was synthesized in these laboratories as described previously [30] and was dissolved in water. Haloperidol was dissolved in 0.01 M tartaric acid. The solutions were freshly prepared in concentrations such that each mouse received a volume of 0.01 ml/g mouse and the rats received 1 ml/kg. Unless otherwise noted, all injections were given intraperitoneally (IP).

Mice were divided into two groups. One group received vehicle (water) and the other received cyclo (Leu-Gly) (50 μg per mouse) injected subcutaneously. One hour later each group was divided into two subgroups. One subgroup of mice received the vehicle (0.01 M tartaric acid) and the other subgroup received haloperidol (1 mg/kg). Mice from each group received their respective injections of drugs and vehicles daily for 21 days. Forty-eight hours after the last injection behavioral response to apomorphine was evaluated as follows. Mice in all the four groups: [vehicle-haloperidol, cyclo (Leu-Gly)- haloperidol, vehicle-vehicle and cyclo (Leu-Gly)-vehicle] were injected IP with apomorphine (1 mg/kg). Fifteen min after apomorphine injection, the motor activity of mice was recorded for 15 min subsequent to a 5 min preambulatory period in a Stoelting activity monitor (The Stoelting Co., Chicago, IL). The activity was expressed as mean counts±SEM. Rectal temperatures of mice were also measured immediately before and 30 min after apomorphine (1 mg/kg) administration using a rectal probe (inserted 2.5 cm into the rectum) and a telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH). The data were expressed as the difference between the temperature reading at 0 and 30 min. The activity and temperature data were analyzed by using Students "t" test (two tailed).

RESULTS

Chronic administration of haloperidol to mice resulted in the development of supersensitivity of dopamine receptors. This supersensitivity was evidenced by the exaggerated response to apomorphine on both the spontaneous motor activity as well as on body temperature in mice. As can be seen in Table 1, apomorphine produced greater locomotor stimulation and greater hypothermia in mice treated chronically with haloperidol compared with mice treated chronically with the vehicle. Chronic treatment with cyclo (Leu-Gly) did not alter apomorphine responses either on spontaneous locomotor activity or the hypothermia in chronically vehicle treated mice. As shown in Table 1, a dose of apomorphine (1 mg/kg) produced a 1.23°C drop in rectal temperature in water-vehicle treated group which was not significantly different from cyclo (Leu-Gly)-vehicle treated group. Administration of apomorphine to chronically haloperidol treated mice resulted in a significantly greater motor activity counts (492±32), compared with the vehicle controls (263±51). Similarly, a greater hypothermia (a drop of 2.11°) was observed in haloperidol treated mice compared with a drop of 1.23°C in vehicle treated mice. Pretreatment with cyclo (Leu-Gly) prevented the development of behavioral supersensitivity of dopamine receptors in chronically haloperidol treated mice. The latter was evidenced by a blockage of the enhanced locomotor and hypothermic effects observed following apomorphine administration. As indicated in Table 1, the apomorphine-induced effects on motor activity and the body temperature of mice from water-vehicle, cyclo (Leu-Gly)-vehicle, and cyclo (Leu-Gly)-haloperidol groups were not different. The apomorphine-induced responses to the water-haloperidol group were, however, significantly ($p<0.05$) different from all the remaining three groups of mice.

DISCUSSION

We have previously shown that cyclo (Leu-Gly), which blocks the development of tolerance and physical dependence on morphine [3, 4, 34], also blocks the dopamine receptor supersensitivity produced as a result of chronic morphinization [3, 20, 21, 27, 30]. In addition to the opiates, long term treatment with neuroleptics also produces hypersensitivity of dopamine receptors as evidenced by behavioral [5, 6, 18, 20, 33] or biochemical measurements [5, 6, 21, 26]. The present studies also indicate that, in mice, behavioral supersensitivity to dopamine agonist apomorphine, the actions of which are thought to reflect stimulation of dopamine recep-

tors [2, 14, 15], is also produced as a result of the long term administration of haloperidol.

Several similarities exist between the actions of opiates and neuroleptic drugs. Comparative actions of the two types of drugs were elegantly summarized by Lal *et al.* [25]. Thus, both drugs produce catalepsy [1], increase dopamine turnover in the brain [1], and block morphine-induced withdrawal aggression, as well as the aggression induced by apomorphine, amphetamine-L-dopa or electroshock administration and hypothermia [25]. Furthermore, tolerance develops to their cataleptic action. Other behavioral similarities were observed when the drugs, morphine or haloperidol, were given chronically to animals and then withdrawn. Cross tolerance to some opiate-neuroleptic actions has been demonstrated [16,28]. These authors concluded that haloperidol blocks dopamine receptors directly. whereas opiates block these receptors indirectly, possibly through trans-synaptic mechanism by which input to the dopaminergic systems is decreased. A similar conclusion was arrived at biochemically by studying the dopamine-sensitive adenylate cyclase of the caudate nucleus of rats treated with morphine or haloperidol [21]. These authors concluded that "haloperidol was a direct effect on the dopamine receptors associated cyclase activity, morphine must act by another mechanism, and that chronic use of either drug produces enhanced dopamine sensitivity."

Even though morphine and neuroleptics show some similarities, there are several differences in the actions of the two drug types. As indicated above, neuroleptics seem to block the dopamine receptor directly, whereas morphine acts by some indirect mechanism. Apomorphine, a direct postsynaptic dopamine receptor agonist prevents morphine catalepsy, but is less effective in blocking haloperidol catalepsy [17,29]. Naloxone antagonizes morphine catalepsy, but has no effect on haloperidol catalepsy [29]. The actions of neuroleptics are blocked by anticholinergics, whereas those of opiates are not [8,17]. Morphine possesses analgesic activity whereas haloperidol does not [25].

Since both the opiates and the neuroleptics on chronic administration produce supersensitivity of brain dopamine receptors, and the opiate induced supersensitivity was blocked by cyclo (Leu-Gly) in mice [30] and rats [3], it was thought that cyclo (Leu-Gly) might also block the dopamine receptor supersensitivity induced by chronic haloperidol treatment. In the present study, the latter was found to be true.

The mechanism by which cyclo (Leu-Gly) inhibits the development of neuroleptic-induced dopamine receptor supersensitivity (receptor proliferation) is not clear at present. It must be noted, however, that receptor proliferation may be related to increased protein synthesis, since protein synthesis inhibitors like cycloheximide or anisomycin can attenuate the enhanced responsitivity to apomorphine induced by short-term attenuation of dopaminergic activity [8]. It is quite possible that attenuation of enhanced response to apomorphine in chronic haloperidol treated mice by cyclo (Leu-Gly) may be related to inhibition of receptor protein synthesis. Such studies are in progress in these laboratories.

In an earlier study [13] with melanocyte stimulating hormone release inhibiting factor (MIF), of which cyclo (Leu-Gly) is an analog, it was found that in humans the severity of signs of tardive dyskinesia was significantly less after multiple oral administration of MIF, even though it was temporary. The effectiveness, however, could be improved by intravenous or intramuscular administration since the amount of MIF reaching the brain after oral administration is not known. The effect of MIF was thought to have been due to the stabilization or binding of some of the dopamine receptor sites to reduce hypersensitivity [13].

Many studies [20, 23, 24, 26, 32] have associated the development of tardive dyskinesia syndrome induced by chronic neuroleptic treatment and drug dependence [17,22] with enhanced dopamine receptor activity in the brain. The latter was blocked by cyclo (Leu-Gly). Thus cyclo (Leu-Gly) and/or related agents might be of value in preventing the development of the symptoms of tardive dyskinesias and development of a drug tolerance-dependence process.

Further studies are in progress to characterize biochemically the effects of cyclo (Leu-Gly) on the changes in ^{3}H-spiroperidol binding in the striata of rats which had been treated chronically with neuroleptic drugs. In addition the effect of cyclo (Leu-Gly) on brain dopamine, synthesis, turnover, release and uptake are being pursued in these laboratories.

ACKNOWLEDGEMENTS

These studies were supported by grants from the Illinois Department of Mental Health and Developmental Disabilities No. 904-02 and USPHS grants DA-02542 and DA-02598. The authors thank Mrs. Cheryl King for her help in the preparation of this manuscript.

REFERENCES

1. Ahtee, L. and I. Kaariainen. The effect of narcotic analgesics on the homovanillic acid content of rat nucleus caudatus. *Eur. J. Pharmac.* **22:** 206–208, 1973.
2. Anden, N. E., A. Robinson, K. Fuxe and T. Hokfelt. Evidence for dopamine receptor stimulation by apomorphine. *J. Pharm. Pharmac.* **19:** 627–629, 1967.
3. Bhargava, H. N. Cyclo (leucylglycine) inhibits the development of morphine-induced analgesic tolerance and dopamine receptor supersensitivity in rats. *Life Sci.* **27:** 117–123, 1980.
4. Bhargava, H. N., R. Walter and R. F. Ritzmann. Development of narcotic tolerance and physical dependence: effects of Pro-Leu-Gly-NH_2 and cyclo (Leu-Gly). *Pharmac. Biochem. Behav.* **12:** 73–77, 1980.
5. Clow, A., A. Theodorou, P. Jenner and C. D. Marsden. Changes in rat striatal dopamine turnover and receptor activity during one year's neuroleptic administration. *Eur. J. Pharmac.* **63:** 135–144, 1980.
6. Clow, A., A. Theodorou, P. Jenner and C. D. Marsden. Cerebral dopamine function in rats following withdrawal from one year of continuous neuroleptic administration. *Eur. J. Pharmac.* **63:** 145–157, 1980.
7. Collier, H. O. J. Supersensitivity and dependence. *Nature, Lond.* **220:** 228–231, 1968.
8. Constentin, J., H. Marcais, P. Protais, M. Baudry, W. de la Baume, M. P. Matres and J. C. Schwarz. Rapid development of hypersensitivity of striatal dopamine receptors induced by alpha methylparatyrosine and its prevention by protein synthesis inhibitors. *Life Sci.* **21:** 307–314, 1977.
9. Costall, B. and R. J. Naylor. On catalepsy and catatonia and the predictability of the catalepsy test for neuroleptic activity. *Psychopharmacologia* **34:** 233–241, 1974.
10. Crane, G. E. Persistent dyskinesia. *Br. J. Psychiat.* **122:** 395–405, 1973.

11. Crane, G. E. and G. Paulson. Involuntary movements in a sample of chronic mental patients and their relation to the treatment with neuroleptics. *Int. J. Neuropsychiat.* **3:** 286–291, 1967.
12. Creese, I., D. R. Burt and S. H. Snyder. Dopamine receptor binding predicts clinical and pharmacological properties of antischizophrenic drugs. *Science* **192:** 481–482, 1976.
13. Ehrensing, R. H., A. J. Kastin, P. F. Larsons and G. A. Bishop. Melanocyte stimulating hormone release inhibiting factor-1 and tardive dyskinesia. *Dis. nerv. Syst.* **38:** 303–307, 1977.
14. Ernst, A. M. Relation between the action of dopamine and apomorphine and their O-methylated derivatives upon the CNS. *Psychopharmacologia* **7:** 391–399, 1965.
15. Ernst, A. M. Mode of action of apomorphine and dexamphetamine on gnawing compulsion in rats. *Psychopharmacologia* **10:** 316–323, 1967.
16. Ezrin-Waters, C. and P. Seeman. Haloperidol-induced tolerance to morphine catalepsy. *Life Sci.* **21:** 419–422, 1977.
17. Ezrin-Waters, C., P. Muller and P. Seeman. Catalepsy induced by morphine or haloperidol: effects of apomorphine and anticholinergic drugs. *Can. J. Physiol. Pharmac.* **54:** 516–519, 1976.
18. Fjalland, B. and I. Moller-Nielsen. Enchancement of methylphenidate-induced stereotypies by repeated administration of neuroleptics. *Psychopharmacologia* **34:** 105–109, 1974.
19. Gianutsos, G., R. B. Drawbaugh, M. D. Hynes and H. Lal. Behavioral evidence for dopaminergic supersensitivity after chronic haloperidol. *Life Sci.* **14:** 887–898, 1974.
20. Gianutsos, G., M. D. Hynes, S. K. Puri, R. B. Drawbaugh and H. Lal. Effect of apomorphine and nigrostriatal lesions on aggression and striatal dopamine turnover during morphine withdrawal. Evidence of dopaminergic supersensitivity in protracted abstinence. *Psychopharmacologia* **34:** 37–44, 1974.
21. Iwatsubo, K. and D. H. Clouet. Dopamine sensitive adenylate cyclase of the caudate nucleus of rats treated with morphine or haloperidol. *Biochem. Pharmac.* **24:** 1499–1503, 1975.
22. Jaffe, J. J. and S. K. Sharpless. In: *The Addictive States,* edited by A. Wikler. Williams and Wilkins, Baltimore, 1968, pp. 226–246.
23. Klawans, H. L. and R. R. McKendall. Observations on the effect of levodopa on tardive, lingual-facial-buccal dyskinesia. *J. Neurol. Sci.* **14:** 189–192, 1971.
24. Klawans, H. L. and H. Rubovits. An experimental model of tardive dyskinesia. *J. Neural Trans.* **33:** 235–246, 1972.
25. Lal, H., G. Gianutsos and S. K. Puri. A comparison of narcotic analgesics with neuroleptics on behavioral measures of dopaminergic activity. *Life Sci.* **17:** 29–34, 1975.
26. Muller, P. and P. Seeman. Brain neurotransmitter receptors after long term haloperidol: dopamine, acetylcholine, serotonin α-noradrenergic and naloxone receptors. *Life Sci.* **21:** 1751–1758, 1977.
27. Puri, S. K. and H. Lal. Effect of dopaminergic stimulation or blockade on morphine withdrawal aggression. *Psychopharmacologia* **32:** 113–118, 1973.
28. Puri, S. K. and H. Lal. Tolerance to the behavioral and neurochemical effects of haloperidol and morphine in rats chronically treated with morphine or haloperidol. *Naunyn-Schmiedebergs Arch. Pharmac.* **282:** 155–170, 1974.
29. Puri, S. K., C. Reddy and H. Lal. Blockade of central dopaminergic receptors by morphine: effect of haloperidol, apomorphine or benztropine. *Res. Commun. chem. pathol. Pharmac.* **5:** 389–401, 1973.
30. Ritzmann, R. F., R. Walter, H. N. Bhargava and L. B. Flexner. Blockage of narcotic induced dopamine receptor supersensitivity by cyclo (Leu-Gly) *Proc. natn. Acad. Sci. U.S.A.* **76:** 5997–5998, 1979.
31. Sesame, H. A., J. Perez-Cruet, G. D. Chiara, A. Tagliamonte, P. Tagliamonte and G. L. Gessa. Evidence that methadone blocks dopamine receptors in the brain. *J. Neurochem.* **19:** 1953–1957, 1972.
32. Sayers, A. C., H. R. Burki, W. Ruch and H. Asper. Neuroleptic-induced hypersensitivity of striatal dopamine receptors in the rat as a model of tardive dyskinesias. Effects of clozapine, haloperidol, loxapine and chlorpromazine. *Psychopharmacologia* **41:** 97–104, 1975.
33. Tarsy, D. and R. J. Baldessarini. Behavioral supersensitivity to apomorphine following chronic treatment with drugs which interfere with the synaptic function of catecholamines. *Neuropharmacology* **13:** 927–940, 1974.
34. Walter, R., R. F. Ritzmann, H. N. Bhargava and L. B. Flexner. Prolyl-leycyl-glycinamide, cyclo (leucylglycine) and derivatives block development of physical dependence on morphine in mice. *Proc. natn. Acad. Sci. U.S.A.* **76:** 518–520, 1979.
35. York, D. H. Dopamine receptor blockade—a central action of chlorpromazine on striatal neurones. *Brain Res.* **37:** 91–99, 1972.