Mennini, T., Bernasconi, S., Manara, L., Samanin, R., Serra, G. (1977) Pharmacol. Res. Commun. 9: 857–862

Pantarotto, C., Belvedere, G., Frigerio, A., Mennini, T., Manara, L. (1976) Eur. J. Drug. Metab. Pharmacokinet. 1:25–34

Samanin, R., Ghezzi, D., Valzelli, L., Garattini, S. (1972) Eur. J. Pharmacol. 19:318–322

Valzelli, L., Garattini, S. (1968) J. Neurochem. 15:259–261

Effect of systemic and intrastriatal injections of haloperidol on striatal dopamine and DOPAC concentrations in rats pretreated by section of nigrostriatal fibres

S. M. WUERTHELE*, K. E. MOORE†, *Department of Pharmacology and Toxicology Michigan State University, East Lansing, Michigan 48824, U.S.A.*

Systemic administration of dopamine receptor antagonists increases, while dopamine agonists decrease the activity of nigrostriatal dopaminergic neurons (Carlsson & Lindqvist 1963; Bunney et al 1973a,b). It has generally been assumed that the changes in nigrostriatal activity induced by systemic administration of dopamine agonists and antagonists result primarily from activation of dopamine receptors located either on dopaminergic cell bodies or dendrites in substantia nigra (autoreceptors), on cell bodies or dendrites postsynaptic to dopaminergic terminals in the striatum (postsynaptic receptors) or on striatal dopaminergic terminals (presynaptic receptors).

According to the presynaptic receptor hypothesis (Christiansen & Squires 1974; Nowycky & Roth 1978; Farnebo & Hamberger 1970; Reimann et al 1979), activation of dopamine receptors located on dopamine nerve terminals or axons in the striatum inhibits synthesis and release of dopamine. On the other hand, stimulation of postsynaptic dopamine receptors has been postulated (Carlsson & Lindqvist 1963) to control nigrostriatal activity by activation of an inhibitory striatonigral feedback loop. That such a loop mediates changes in nigrostriatal activity produced by systemic administration of dopamine agonists and antagonists, has recently been questioned. After kainic acid-induced destruction of the striatal cell bodies forming this loop, nigrostriatal neurons still respond to dopamine agonists with decreased, and to dopamine antagonists with increased activity, as estimated either by changes in striatal dihydroxyphenylacetic acid (DOPAC) concentrations, or by the α-methyl-*p*-tyrosine-induced decline of striatal dopamine concentrations (Di Chiara et al 1977; Wuerthele & Moore 1979). Experiments with section or electrolytic lesions of the nigrostriatal fibres (Bedard & Larochelle 1973; Garcia-Munoz et al 1977) also suggest that a feedback loop is not essential for changes in nigrostriatal activity induced by dopamine agonists and antagonists. The following report supports this conclusion and presents evidence that the increases in nigrostriatal activity produced by the systemic administration of dopamine antagonists are only partially due to the blockade of presynaptic striatal receptors.

Male Sprague-Dawley rats (200–300 g, Spartan Research Animals, Haslett, Michigan) were anaesthetized with Equithesin (3 ml kg^{-1}). Sections of the striatonigral path was effected by lowering a 2·2 mm wide stainless steel blade to the base of the skull 2·2 mm anterior to the intra-aural line, with the medial edge of the blade 1·8 mm lateral to the midline (König & Klippel 1963). Hemitranssections were made by slowly moving this knife laterally from the midline 4·8 mm. In some animals, 23 gauge cannula guides were permanently implanted into the striatum (2·0 mm anterior to Bregma, ± 3·0 mm lateral from the midline and 4·3 mm ventral from the dura (Pellegrino & Cushman 1967). Injections were made through these cannula guides by inserting 30 gauge injector cannulae into the implanted guides, so that the tip extended 1 mm below the tip of the guide cannula. Drugs were injected over 2 min from a 5 μl syringe mounted on an infusion pump and connected to the cannulae by short pieces of polyethylene tubing. Animals were decapitated, and brains rapidly removed and dissected on a thermoelectric cold plate. Pieces of tissue containing the striatum and substantia nigra were frozen on dry ice, sliced and further dissected with the aid of a stereoscope. Striatal and nigral concentrations of dopamine and DOPAC were measured by a radioenzymatic method (Umezu & Moore 1979). Nigral glutamic acid decarboxylase (GAD) activity was measured by a modification of the method of Kanazawa et al (1976). Significance of the differences between means were tested using two-way analysis of variance (Sokol & Rohlf 1969). Regression analysis was used to test the correlation between nigral GAD activities and increases in striatal DOPAC concentrations (Table 3). Injectable haloperidol (Haldol) and its vehicle were supplied by Dr J. Plostnieks, McNeil Laboratories, Ft. Washington, Pennsylvania. Sulpiride was obtained from Ravizza Research Laboratories, Milan, Italy.

Because the nigrostriatal and striatonigral fibres lie in close proximity, it is not possible to make completely selective lesions of the descending striatonigral fibres. Therefore, only rats in which sectioning produced less

* Present address: Department of Pharmacology, C236 University of Colorado Health Sciences Center, 4200 E. Ninth Ave. Denver, CO 80262, U.S.A.

† Correspondence.

Table 1. Striatal dopamine and DOPAC concentrations and nigral GAD activity in rats given hemistranssections, section of the nigrostriatal pathway, or section and systemic haloperidol. Hemitranssections or sections were made at the anterior tip of the substantia nigra (+ 4·4 mm anterior to the intra-aural line, midline to 4·0 mm, and 1·8 to 4·0 mm lateral to the midline, respectively; König & Klippel 1963). Seven to eleven days later, all animals were killed. Sectioned animals were given haloperidol (0·1 mg kg^{-1}, i.p.) 1 h before death.

Group	Hemitrans-sections (n = 7)	Ablation (n = 6)	Ablation + systemic haloperidol (n = 7)
Nigral GAD activity (% of intact side)	17 ± 4%	51 ± 5%	51 ± 4%
Striatal dopamine (ng mg^{-1} protein)			
Intact	98·22 ± 10·9	91·99 ± 3·9	93·51 ± 6·8
Sectioned	12·46 ± 4·2	83·86 ± 5·2	120·20 ± 14·9
% of intact:	13·1%	92%	128%
Striatal DOPAC (ng mg^{-1} protein)			
Intact	7·98 ± 0·29	7·95 ± 1·0	25·98 ± 1·5[a]
Sectioned	1·19 ± 0·33	7·13 ± 1·1	34·65 ± 4·6[b]
% of intact:	18·3%	90%	130%

[a] Significantly greater than non-drug-treated animals; ($P \leq 0.05$)
[b] Significantly greater than intact striatum; ($P \leq 0.05$)

than 10% depletion of striatal dopamine compared with the contralateral striatum (indicating that at least 90% of the nigrostriatal fibres remained intact) and approximately 50% depletion of nigral GAD activity were included in these studies (Table 1).

Hemitranssections do not completely eliminate nigral GABA or GAD activity. For example, it has been reported that hemitranssections leave from 25 to 50% residual GAD activity in this structure (Gale et al 1977; Kanazawa et al 1977; Kim et al 1971). In the studies summarized in Table 1, hemitranssections left an average of 17% residual nigral GAD activity. Therefore, 83% of the total activity can be attributed to descending neurons (e.g., striatonigral fibres) which degenerated following the lesion. The 51% depletion of total activity observed after sectioning in (Table 1) then represents a 59% destruction of the feedback loop. Since these depletions of total GAD activity are greater than those reported previously (Gale et al 1977; Kanazawa et al 1977; Kim et al 1971), this estimate of feedback loop destruction is conservative.

In the sectioned group, nigrostriatal fibres remained intact, since no significant alteration was observed in dopamine concentrations ipsilateral to the cut. In these animals, systemic administration of haloperidol increased striatal DOPAC concentrations on both the sectioned and the contralateral sides. Like the kainic acid experiments, these data indicate that a striatonigral feedback loop is not essential for drug-induced alterations in nigrostriatal activity. Doses of haloperidol known to produce nearly maximal increases in striatal DOPAC concentrations were used to maximize the chances of observing a response to the drug in the absence of the feedback loop neurons, or possibly to observe a decrease in the maximal response if the feedback loop is indeed responsible for a part of the response to antipsychotics. Furthermore, when sectioning (Table 1) or kainic acid (Di Chiara et al 1977) are used to destroy the feedback loop, the response to haloperidol is significantly greater on the lesioned than on the non-lesioned side. Since destruction of the feedback loop results in an exaggerated response to neuroleptics, these drugs may act presynaptically to increase, but postsynaptically to decrease, dopaminergic nerve activity. This is contrary to the hypothesis that dopamine antagonists increase nigrostriatal neuronal activity through a feedback loop.

Table 2. Striatal and nigral DOPAC concentrations following intrastriatal administration of dopamine antagonists. Rats were implanted bilaterally with permanent striatal cannula guides. Two to four days later, drugs dissolved in 2·0 μl vehicle were injected into the right striatum, and 2·0 μl vehicle (Haldol vehicle for haloperidol, 0·3% tartaric acid for sulpiride) was injected into the left striatum. Animals were killed 15 (sulpiride) or 30 min (haloperidol) after injection.

Treatment	Haloperidol (5 μg)	Sulpiride (10 μg)
Striatal DOPAC (ng mg^{-1} protein)		
Drug	21·40 ± 0·4[a]	13·43 ± 1·0[a]
Vehicle	14·03 ± 0·9	9·18 ± 1·1
% Control	152%	146%
n =	(24)	(20)
Nigral DOPAC (ng mg^{-1} protein)		
Drug	2·85 ± 0·3	1·57 ± 0·1
Vehicle	2·12 ± 0·4	1·75 ± 0·2
% Control	74%	90%
n =	(12)	(15)

[a] Significantly different from control (left) side; ($P \leq 0.05$)

Both kainic acid and sectioning experiments are open to the criticism that these treatments may not produce complete destruction of the feedback loop, and that remaining neurons support normal function. For example, remaining dopamine receptors might become supersensitive to the transmitter. However, histological studies (Wuerthele et al 1978) suggest that it is unlikely that at least kainic acid leaves any postsynaptic cell bodies intact, and the exaggerated response to neuroleptics seen with both treatments cannot easily be attributed to a small number of residual neurons.

If there is a striatal dopamine receptor mechanism controlling dopamine release from nigrostriatal terminals, then striatal injections of dopamine antagonists should produce changes in nigrostriatal activity at least qualitatively similar to those observed after systemic drug administration. The results of intrastriatal injections of dopamine antagonists shown in Table 2 indicate that this is the case. Maximally effective injections of haloperidol and sulpiride increased striatal DOPAC concentrations 153 and 146%, respectively. These are similar to results obtained by intrastriatal injections of another antagonist, trifluperidol (Racagni et al 1978).

Table 3. Striatal dopamine and DOPAC concentrations and nigral GAD activity following intrastriatal haloperidol in sectioned animals. Data from individual animals have been placed into arbitrary groups based on % GAD depletion. All rats received bilateral striatal cannula implants and section of nigrostriatal fibres on the right side (R). One week later, they were given intrastriatal injections of haloperidol (5 μg/2 μl) over 2 min into the right striatum, haloperidol (Haldol) vehicle (2 μl over 2 min) into the left striatum, and were killed 30 min later. Values of striatal dopamine and nigral GAD represent the sectioned side as a % of the control side. Actual values of striatal DOPAC from both sectioned and untreated sides are listed. The n values, means and standard errors listed are cumulative values incorporating the animals from each successive group. Control dopamine concentrations are 86·38 ± 3·54 ng mg^{-1} protein, and control GAD activity is 265 ± 17 counts min^{-1} μg^{-1} protein per 15 min.

	Nigral GAD activity		Striatal dopamine		Striatal DOPAC (ng mg^{-1} protein)		
Cum. n:	% Cont:	Cum. %:	% cont.	Cum. %:	L	R	Cum. %:
5	25		78		6·70	9·96	
	33		67		6·34	9·92	
	34		86		6·10	10·03	
	39		83		6·72	6·02	
	41		67		5·81	9·45	
		34 ± 3		76 ± 4	6·33 ± 0·17	9·08 ± 0·77[a]	144 ± 14
	50		96		3·18	6·27	
	52		89		5·58	8·09	
	53		95		7·41	11·06	
	58		93		7·20	5·97	
10	59		89		9·00	8·71	
		44 ± 4		84 ± 3	6·40 ± 0·47	8·61 ± 0·62[a]	140 ± 12
14	61		109		5·83	8·90	
	62		82		7·96	11·10	
	64		79		5·78	7·84	
	64		63		7·51	5·70	
		50 ± 4		84 ± 3	6·51 ± 0·36	8·54 ± 0·52	142 ± 9

[a] Significantly greater than control (L); ($P \leq 0·05$)

To test whether the response to local administration of neuroleptics is mediated presynaptically, haloperidol was injected into the striatum of animals whose nigrostriatal fibres were destroyed (Table 3). This treatment increased striatal DOPAC concentrations even though the nigrostriatal fibres were at least partially disrupted. Because of the difficult technical nature of these experiments, these data are presented by individual animal, according to the degree of feedback loop destruction (i.e., nigral GAD activity remaining). Regression analysis indicates that there is no correlation between nigral GAD activity and the percent increase in striatal DOPAC concentrations in the groups shown ($r = 0·027$). Compensatory changes in any remaining feedback loop neurons probably do not account for the release of dopamine observed, since the response to intrastriatal haloperidol is independent of the degree of feedback loop destruction. These data support the argument that dopamine release is controlled, at least in part, via presynaptic mechanisms, but they do not completely eliminate the possibility of a short feedback loop of interneurons, or an axon collateral arrangement. Nevertheless, nigrostriatal neurons still respond to agonists and antagonists after kainic acid, a treatment that appears to destroy all postsynaptic cells (Di Chiara et al 1977; Wuerthele & Moore 1979). Furthermore, because of the diffuse nature of the dopaminergic innervation to the striatum, a short feedback loop system would be expected to form many axoaxonic synapses, and these are very rare in striatum (Kemp & Powell 1971), the predominant type being axodendritic.

The fact that the neuronal response to systemically administered haloperidol is much greater than to the locally administered drug, and that systemic, but not local administration increases nigral DOPAC concentrations (Table 2; Wuerthele et al 1979), indicate that the intrastriatal and systemic routes are not equivalent. This may be the result of inadequate distribution of locally administered haloperidol throughout the striatum. On the other hand, it may be that the action of systemically administered dopaminergic antagonist affects nigrostriatal activity via afferents to substantia nigra other than the striatonigral fibres.

In summary, these experiments support the hypothesis that presynaptic mechanisms influence dopamine release in the striatum.

This work was supported by USPHS Grant MH 13174. S. M. Wuerthele is a predoctoral student supported by USPHS Training Grant GM07392.

November 5, 1979

REFERENCES

Bedard, P., Larochelle, L. (1973) Exp. Neurol. 41: 314–322

Bunney, B. S., Aghajanian, G. K., Roth, R. H. (1973a) Nat. New Biol. 245: 123–125

Bunney, B. S., Walters, J. R., Roth, R. H., Aghajanian, G. K. (1973b) J. Pharmacol. Exp. Ther. 185: 560–571

Carlsson, A., Lindqvist, M. (1963) Acta. Pharmacol. Toxicol. 20: 140–144

Christiansen, J., Squires, R. F. (1974) J. Pharm. Pharmacol. 26: 367–369

Di Chiara, G., Porceddu, M. L., Fratta, W., Gessa, G. L. (1977) Nature (London) 267: 270–272

Farnebo, L. O., Hamberger, B. (1971) Acta Physiol. Scand. (Suppl. 371): 35–44

Gale, K., Hong, Y. S., Guidotti, G. (1977) Brain Res. 136: 371–375

Garcia-Munoz, M., Nicolaou, N. M., Tullock, I. F., Wright, A. K., Arbuthnott, G. W. (1977) Nature (London) 265: 363–365

Kanazawa, I., Emson, P. C., Cuello, A. C. (1977) Brain Res. 119: 447–453

Kanazawa, I., Iversen, L. L., Kelly, J. S. (1976) J. Neurochem. 27: 1267–1269

Kemp, J. M., Powell, T. P. S. (1971) Phil. Trans. R. Soc. London Ser. B 262: 403–412

Kim, J. S., Bak, I. J., Hassler, R., Okada, Y. (1971) Exp. Brain Res. 14: 95–104

König, J. F. R., Klippel, R. A. (1963) The Rat Brain, Robert E. Kreiger Pub. Co. Inc., New York

Nowycky, M. C., Roth, R. H. (1978) Prog. Neuropsychopharmacol. 2: 139–158

Pellegrino, L. J., Cushman, A. (1967) A Stereotaxic Atlas of the Rat Brain, Appleton-Century-Crofts, New York

Racagni, G., Groppetti, A., Parenti, M., Bugatti, A., Bruno, F., Maggi A., Cattabeni, F. (1978) Life Sci. 23: 1757–1762

Reimann, W., Zumstein, A., Jackish, R., Starke, K., Hertig, G. (1979) N.S. Arch. Pharmacol. 306: 53–60

Sokol, R. R., Rohlf, R. J. (1969) Biometry, W. H. Freeman Co., San Francisco

Umezu, K., Moore, K. E. (1979) J. Pharmacol. Exp. Ther. 208: 49–56

Wuerthele, S. M., Lovell, K. L., Jones, M. Z., Moore, K. E. (1978) Brain Res. 149: 489–497

Wuerthele, S. M., Moore, K. E. (1979) J. Pharm. Pharmacol. 31: 180–182

Chronic lithium prevents reserpine-induced supersensitivity of adenylate cyclase

MIRA HERMONI, BERNARD LERER, RICHARD P. EBSTEIN*, ROBERT H. BELMAKER, *Jerusalem Mental Health Center-Ezrath Nashim, P.O.B. 140, Jerusalem, Israel*

Pert et al (1978) reported that chronic oral 0·2% lithium (Li) can block the development of increases in dopamine receptor number in the rat caudate nucleus induced by chronic haloperidol. Li plasma concentrations were 0·8–1·0 mM. These authors suggested that Li may act therapeutically by stabilizing receptor number changes. Treiser & Kellar (1979) found that chronic 0·2% oral Li, yielding plasma concentrations of 0·8–1·1 mM, was able to prevent the development of reserpine-induced increases in rat cortex β-adrenoceptor number. Chronic Li alone was found by both Rosenblatt et al (1979) and Treiser & Kellar (1979) to cause a slight decrease in β-adrenoceptor number. Since noradrenaline-stimulated adenylate cyclase activity and radioligand measured β-adrenoceptor number are closely parallel in many systems (Lefkowitz & Williams 1978), we decided to investigate the effect of chronic Li on reserpine-induced increases in rat cortical noradrenaline-sensitive adenylate cyclase activity (Baudry et al 1976).

Male albino rats (Sabra strain, 200–250 g) obtained from the Hebrew University were used. Rat food containing 0·15% LiCl was prepared by grinding regular rat pellets to a fine powder and thoroughly mixing with LiCl. The Li-treated rats were maintained on this diet for 3–5 weeks and then received a series of 4 days of i.p. injections of reserpine or 0·9% NaCl (saline) while continuing to receive Li orally. Reserpine dose was 5 mg kg^{-1} on the first day and 2·5 mg kg^{-1} on the following 3 days. Animals were decapitated 24 h after the last reserpine dose and the brains rapidly removed and placed on a precooled cutting block at 4–8°C. Carotid blood at death was taken for Li determination, which averaged 0·4 mM. Cortical 1 mm cubes were prepared using a McIllwain Tissue Chopper. The tissue was incubated in Krebs Ringer bicarbonate solution containing glucose as described by Kakiuchi & Rall (1968) with continuous gassing (95% O_2 and 5% CO_2). After 20 min preincubation the Ringer solution was changed and the tissue incubated for an additional 10 min. At the end of the second incubation period noradrenaline was added for an additional 12 min. The reaction was stopped by addition of 95% ethanol. The tissue was homogenized in ethanol and an aliquot evaporated down and the cyclic (c)AMP assayed using a kit supplied by the Radiochemical Centre Amersham, U.K. Protein was determined by the procedure of Lowry et al (1951).

The results shown in Table 1 demonstrate that chronic Li pre-treatment can prevent reserpine-induced increases in noradrenaline-induced cAMP accumulation. This is the first demonstration that Li prevents receptor supersensitivity as measured by adenylate cyclase activity. The results parallel those of Treiser &

* Correspondence.

Supported in part by a grant from The Joint Research Fund of the Hebrew University and Hadassah.