Effects of dopaminergic antagonists on striatal DOPAC concentrations and α-methyl-*p*-tyrosine-induced decline of dopamine following intrastriatal injections of kainic acid

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

The activity of nigrostriatal dopaminergic neurons increases following administration of dopamine antagonists and decreases following dopamine agonists (Bunney et al 1973a, b). 3,4-Dihydroxyphenylacetic acid (DOPAC), the major acid metabolite of dopamine, has been used as a biochemical index of dopaminergic nerve activity. For example, the concentration of DOPAC in the striatum increases after the administration of dopamine antagonists and decreases after dopamine agonists (Roth et al 1976).

The effects of these drugs on dopaminergic activity led to the concept of a neuronal feedback control for nigrostriatal neurons (Carlsson & Lindqvist 1963). It is currently believed that nigrostriatal neurons exert a tonic inhibition of acetylcholine-containing interneurons within the striatum. These interneurons, in turn, either directly or indirectly inhibit the activity of GABAergic striatal neurons whose descending axons terminate on dopaminergic cell processes in substantia nigra. Inhibitory action by the transmitter released here completes a negative feedback loop. Increased release of dopamine in the striatum therefore initiates compensatory inhibition of nigrostriatal neurons through the feedback loop. Blockade of any element within this loop causes the release of these neurons from this tonic inhibition and thereby increases their activity. There is biochemical, anatomical and electrophysiological evidence for this hypothesis. Dopamine and dopaminergic agonists inhibit striatal cholinergic neurons, as evidenced by reduced acetylcholine release and turnover, whereas dopamine antagonists have opposite effects (Ladinsky et al 1978: Costa & Cheney 1978). GABA-containing neurons located in the caudate nucleus and putamen project to nigrostriatal neurons in the zona reticulata of substantia nigra (Kim et al 1971), and application of GABA into the nigra inhibits nigrostriatal firing (Feltz 1971).

There is, however, evidence that a negative feedback loop is not necessary for regulation of nigrostriatal activity (Groves et al 1975). Lesions of descending striatonigral neurons do not prevent haloperidol-induced increases in striatal DOPAC and homovanillic acid concentrations (Bedard & Larochelle 1973; Garcia-Munoz et al 1977). Similar results have been obtained following destruction of striatal cell bodies with the neurotoxin kainic acid (DiChiara et al 1977; Wuerthele et al 1977). The following report confirms these results with additional dopamine antagonists, and presents evidence which suggests that DOPAC concentrations may not accurately reflect dopaminergic activity when dopamine terminals are exposed to the toxic effects of kainic acid.

Male Sprague-Dawley rats (Spartan Research Animals, Haslett, MI) 250–275 g, were anaesthetized with Equithesin (3 ml kg^{-1}) and injected with 2·5 μg kainic acid in 2·0 μl saline into the right striatum (stereotaxic coordinates: AP 2·0 LAT 3·0, DV 5·0; Pellegrino & Cushman 1967). Seven days later they were injected with either saline, apomorphine HCl (0·5 mg kg^{-1}, s.c.), piribedil mesylate (30 mg kg^{-1}, i.p.), haloperidol (0·5 mg kg^{-1}, i.p.), thioridazine HCl (10 mg kg^{-1}, i.p.), clozapine (40 mg kg^{-1}, i.p.) or sulpiride (40 mg kg^{-1}, i.p.) and killed 30 min (apomorphine), 1 h (piribedil, haloperidol and thioridazine), 2 h (clozapine), or 3 h (sulpiride) later. These times were chosen to coincide with maximal drug effects on DOPAC concentrations. Striata were dissected on ice, and striatal DOPAC concentrations were measured by a modification of the fluorometric method of Westerink & Korf (1976). Kainic acid-

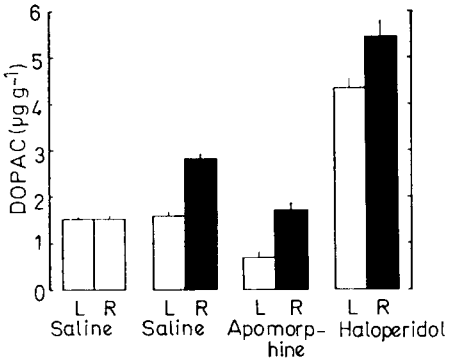

FIG. 1. Effects of apomorphine and haloperidol on concentrations of DOPAC in control and kainic acid-lesioned striata. Rats received a microinjection of saline or kainic acid into the right striatum. Seven days later they received systemic injections of apomorphine (0.5 mg kg^{-1}, s.c.) or haloperidol (0·5 mg kg^{-1}, i.p.) and were killed 30 (apomorphine) or 60 min (haloperidol) later; other animals received appropriate systemic injections of saline. The height of each column represents the mean DOPAC concentration and the vertical line 1 s.e. Solid columns represent kainic acid-treated striata; open columns represent control or saline-treated striata. The number of animals in each group were: saline-saline, 15; saline-kainic acid, 36; apomorphine-kainic acid, 11; haloperidol-kainic acid, 15.

* Correspondence.

induced destruction of striatal cholinergic neurons was confirmed by the assay of choline acetyltransferase (ChAT) activity (Fonnum et al 1974); data shown are from animals with ChAT activities in kainic acid-treated striata ranging from 9·2 to 20·4% of contralateral control striata. Additional kainic acid-treated rats were injected with either saline or haloperidol (0·5 mg kg^{-1}, i.p.) and with α-methyltyrosine methyl-ester (AMPT; 250 mg base kg^{-1}). These animals were killed at 0, 1 or 2 h after receiving AMPT and 3h after haloperidol. Striatal dopamine was measured by a modification (Moore & Phillipson 1975) of the radioenzymatic method of Cuello et al (1973). All data were analysed using two-way analysis of variance (Sokol & Rohlf 1969).

In saline-pretreated rats (second pair of columns in Fig. 1) intrastriatal injections of kainic acid increased the striatal DOPAC concentration on the side of the injection but did not alter the concentration of this metabolite in contralateral striata. That is, the concentration of DOPAC in left striata was not different from the DOPAC concentration in the striata of rats that had received only a unilateral intrastriatal injection of saline (first pair of columns in Fig. 1). As expected, apomorphine reduced the concentrations of DOPAC in control striata. Unexpectedly, apomorphine also reduced DOPAC in kainic acid-injected striata. Similar results were obtained 1 h after an injection of another dopamine agonist, piribedil (30 mg kg^{-1}, data not shown). Conversely, haloperidol increased the DOPAC concentrations in control striata, but caused an even greater increase on the kainic acid side. Similar results were obtained with other dopaminergic antagonists (Table 1); thioridazine, clozapine and sulpiride all increased the DOPAC concentrations in intact striata, and caused an even greater increase in kainic acid-lesioned striata.

To determine if increased concentrations of DOPAC in kainic acid-lesioned striata actually represent increased dopaminergic nerve activity, another biochemical index of dopamine turnover was determined. The decline of striatal dopamine after AMPT is depicted in Fig. 2. Haloperidol increased the rate of decline of dopamine in both control and kainic acid-lesioned striata. Nevertheless, in contrast to the results of experiments in which DOPAC was measured, no differences in the AMPT-induced decline of dopamine were found between lesioned and non-lesioned striata of either saline or haloperidol-treated rats.

Table 1. Effects of dopamine antagonists on striatal DOPAC concentrations in control and kainic acid-lesioned striata.

Treatment	Dose (mg kg^{-1})	N	Striatal DOPAC (μg g^{-1}) Control side	Kainic acid side
Saline		6	1·59 ± 0·10	2·84 ± 0·10
Thioridazine	10	8	3·20 ± 0·30	5·33 ± 0·49
Clozapine	40	13	4·26 ± 0·33	5·94 ± 0·37
Sulpiride	40	11	3·21 ± 0·22	8·10 ± 0·30

Seven days after unilateral intrastriatal injections of kainic acid rats received an i.p. injection of saline or a dopamine antagonist. DOPAC concentrations in both control and kainic acid-lesioned striata were significantly greater (P <0·01) in all dopamine antagonist-treated animals than in saline-treated animals. Furthermore, in all treatment groups the DOPAC concentration was significantly higher (P <0·01) in the kainic acid side than in the control side.

Consistent with the feedback loop hypothesis and with previous results obtained by many others, apomorphine decreased and haloperidol increased the concentrations of DOPAC in control striata. Intrastriatal kainic acid, which destroys the feedback loop, should attenuate the effects of these drugs. Unexpectedly, the haloperidol-induced increase in DOPAC concentration was actually enhanced on the kainic acid-treated side. This has also been noted by Di Chiara et al (1977). This effect was not unique to the butyrophenone neuroleptics but also occurred with other chemical classes of dopamine antagonists. These results suggest that the feedback loop is not essential for dopamine antagonist-induced changes in nigrostriatal dopaminergic nerve activity. This is also supported by the fact that haloperidol was equally effective in enhancing the AMPT-induced decline of dopamine in control and kainic acid-treated striata.

Kainic acid treatment alone significantly increased DOPAC concentrations in the striatum. This might indicate that the activity of dopaminergic nigrostriatal neurons increases as a consequence of the disruption of tonic feedback loop inhibition. However, when the decline of dopamine following AMPT-administration

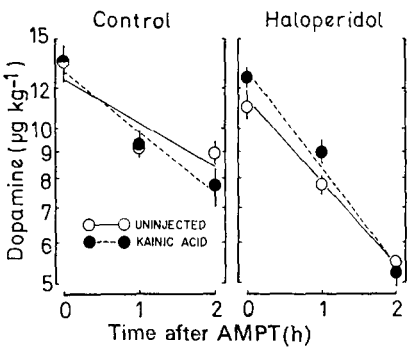

FIG. 2. Effect of haloperidol on striatal dopamine following α-methyltyrosine. All rats were injected unilaterally with kainic acid in the right striatum. Seven days later rats were injected i.p. with haloperidol (0·5 mg kg^{-1}) and AMPT (250 mg kg^{-1}). Animals were killed 3 h after receiving haloperidol and either 0, 1 or 2 h after receiving AMPT. The rate constants for decline of dopamine in control animals are 0·26 and 0·18 for treated and untreated sides, respectively, and in animals receiving haloperidol 0·43 and 0·36 for treated and untreated sides, respectively. Slopes were compared by regression analysis.

is used as an index of dopaminergic nerve activity, no difference between values in control and kainic acid-lesioned striata was observed. Using another index of dopamine nerve activity, Biggio et al (1978) failed to find a significant increase in accumulation of dopa in kainic acid-treated striata following the inhibition of aromatic L-amino acid decarboxylase.

An alternative explanation for increases in striatal DOPAC concentrations is that the extraneuronal enzyme COMT might be lowered by kainic acid treatment, shunting more dopamine to intracellular metabolism and thus raising DOPAC concentrations. It has been demonstrated, however, that kainic acid treatment increases COMT activity (Schwartz & Coyle 1977; Kelly & Moore 1977). If anything, these DOPAC values are conservative estimates of dopamine release.

Antidromic activation of the medial forebrain bundle increases the concentration of DOPAC in substantia nigra in a frequency-dependent manner (Korf et al 1977). Increased nigral DOPAC concentrations are also observed following systemic administration of haloperidol (Wuerthele & Moore unpublished observations). These results suggest that nigrostriatal activity is associated with dopamine release in substantia nigra as well as at striatal terminals. Thus, if kainic acid-induced destruction of the feedback loop releases nigrostriatal neurons from tonic inhibition, kainic acid treatment should increase DOPAC concentrations in substantia nigra as well as in the striatum. This does not occur. Nigral DOPAC concentrations were measured in rats 7 days after unilateral intrastriatal injections of kainic acid (2·5 μg/2·0 μl saline) using a radioenzymatic method (Umezu & Moore 1979). Nigral DOPAC concentrations on the kainic acid-treated side were not significantly different from those on the contralateral side (3·13 $\pm$ 0·5 vs 3·29 $\pm$ 0·48 ng mg^{-1} protein, respectively; n = 6). This failure of kainic acid to increase nigral DOPAC concentrations suggests that such increases observed in the striatum (Table 1) represent a local effect of kainic acid.

These results suggest that under certain circumstances DOPAC is not necessarily equivalent to other indices of dopaminergic nerve activity. When striatal architecture remains intact, DOPAC concentrations parallel dopaminergic nerve activity. For example, electrical stimulation of nigrostriatal neurons increases DOPAC whereas acute lesions of these nerves decreases the striatal concentration of this metabolite (Roth et al 1976). However, kainic acid causes neuronal damage near the site of its injection, including atrophy, demyelination (Wuerthele et al 1978) and loss of dopamine as measured biochemically (Friedle et al 1978) and histochemically (Meibach et al 1978). This suggests that the increased DOPAC concentrations in kainic acid-treated striata may be due to the inability of damaged or degenerating nerve terminals to protect vesicular dopamine stores from intracellular monoamine oxidase.

This work was supported by USPHS grant MH13174. S. M. Wuerthele is a predoctoral student supported by USPHS Training Grant GM07392.

October 10, 1978

REFERENCES

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

Biggio, G., Casu, M., Corda, M. G., Dibello, C., Gessa, G. L. (1978) Science 200: 522–554

Bunney, G. S., Aghajanian, G. K., Roth, R. H. (1973a) Nature (London) 243: 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

Costa, E., Cheney, D. L. (1978) in: Garattini, S., Pujol, J. F, Samanin, R. (eds), Interactions Between Putative Neurotransmitters in the Brain, Raven Press, New York, pp. 23–38

Cuello, A. C., Hiley, R., Iversen, L. L. (1973) J. Neurochem. 21: 1337–1340

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

Feltz, P. (1971) Can. J. Physiol. Pharmacol. 49: 1113–1115

Fonnum, F., Grofova, I., Rinvik, E., Storm-Mathisen, J., Walberg, F. (1974) Brain Res. 71: 77–92

Friedle, N. M., Kelly, P. H., Moore, K. E. (1978) Br. J. Pharmacol. 63: 151–158

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

Groves, P. M., Wilson, C. J., Young, S. J., Rebec, G. V. (1975) Science 190: 522–529

Kelly, P. H., Moore, K. E. (1977) Pharmacologist 19: 225

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

Korf, J., Zieleman, M., Westerink, B. H. C. (1977) Brain Res. 120: 184–187

Ladinsky, H., Consolo, S., Bianchi, S., Ghezzi, D., Samanin, R. (1978) in: Garattini, S., Pujol, J. F., Samanin, R. (eds), Interactions Between Putative Neurotransmitters in the Brain, Raven Press, New York, pp. 3–21.

Meibach, R. C., Brown, L., Brooks, F. H. (1978) Brain Res. 148: 219–223

Moore, K. E., Phillipson, O. T. (1975) J. Neurochem. 25: 289–294

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

Roth, R. H., Murrin, L. C., Walters, J. R. (1976) Eur. J. Pharmacol. 36: 163–171

Schwartz, R., Coyle, J. T. (1977) Brain Res. 127: 235–249

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

Umezu, K., Moore, K. E. (1979) J. Pharmacol. Exp. Ther., In the press

Westerink, B. H. C., Korf, J. (1976) Eur. J. Pharmacol. 38: 281–291

Wuerthele, S. M., Kelly, P. H., Moore, K. E. (1977) Pharmacologist 19: 223

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