J. Pharm. Pharmacol. 1982, 34: 820–822
Communicated March 23, 1982

0022–3573/82/120820–03 $02.50/0

Effect of electrical stimulation of nigrostriatal dopaminergic neurons on utilization of exogenous L-dopa in rat corpus striatum

ELDAD MELAMED*, NACHUM DAFNI, *Laboratory of Clinical Neurochemistry, Department of Neurology, Hadassah University Hospital, Jerusalem, Israel, and the Department of Neurobiology and Anatomy, University of Texas School of Medicine, Houston, Texas, 77025, U.S.A.*

The rates of synthesis and release of dopamine (DA) by striatal DA nerve-terminals are largely determined by the firing activity of DA neurons in the substantia nigra (Korf 1979). Thus, when impulse flow is enhanced in the nigrostriatal projection such as during electrical stimulation, there is an accelerated release of DA synthesized from tyrosine and the dopa formed from tyrosine hydroxylation; this is manifested biochemically by elevated striatal levels of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) with essentially unchanged DA concentrations (Korf et al 1976; Roth et al 1976). While hyperactive DA neurons increase formation and release of DA from endogenous tyrosine and dopa, it is of interest whether they are also capable of utilizing more exogenously administered precursors. There is some evidence indicating that exogenous tyrosine may increase DA synthesis and release but only by rapidly firing and not by resting DA neurons; this effect may be linked to activation of tyrosine hydroxylase (Scally et al 1977; Melamed et al 1980a). In contrast, systemic L-dopa enhances striatal DA synthesis and release even by non-activated DA neurons, since dopa decarboxylase is not a rate-limiting enzyme in the biosynthesis of catecholamines (Melamed et al 1980b; Hefti & Melamed 1981b). However, it remains unknown whether acceleration of impulse flow is associated with increased utilization of exogenous L-dopa by DA neurons. This question is important concerning the mechanism of action of L-dopa in Parkinson's disease and mainly the striatal locus of its enzymatic decarboxylation to DA after degeneration of DA terminals. Clinical and experimental studies indicate that when the nigrostriatal projection is partially destroyed, the remaining neurons become hyperactive and synthesize and release more DA from endogenous precursors than do neurons in an intact system (Agid et al 1973; Bernheimer et al 1973; Hefti et al 1980). On that basis, it was hypothesized that L-dopa's efficacy in Parkinsonism depends on its accelerated utilization by the surviving hyperactive DA neurons which might be capable of converting exogenous L-dopa to DA in amounts adequate to correct the deficient DA neurotransmission despite their markedly small numbers (Hornykiewicz 1974).

* Correspondence.

We therefore examined in rats undergoing electrical stimulation of the substantia nigra whether activation of DA neurons indeed enhances the synthesis and subsequent release of DA from exogenous L-dopa in the corpus striatum.

Method

Male albino rats (Hebrew University strain) 180–200 g were anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p; Korf et al 1976) and placed in a Kopf stereotaxic device. Twisted bipolar electrodes consisting of two 60 μm nichrome wires insulated with diamel except for the tips, were implanted stereotaxically into the left substantia nigra (A 1950, 2 mm lateral, −2·2 mm dorsal, according to the atlas of Konig & Klippel). The electrodes were fixed to the skull with acrylic dental cement. At the end of the surgical procedure, rats were removed from the stereotaxic device and placed in cages. Animals received injections of α-methyldopahydrazine (carbidopa, 100 mg kg^{-1} as a fine suspension in 0·9% NaCl, (saline) i.p.) followed 60 min later by L-dopa (50 mg kg^{-1}, dissolved in saline, i.p.). Carbidopa, a peripheral blocker of dopa decarboxylase, was given to inhibit the enzyme localized in endothelial cells of cerebral capillaries and to eliminate the fraction of the dopa-induced elevations in striatal DA, HVA and DOPAC that derives from decarboxylation of L-dopa in the microvessels (Melamed et al 1980b). Immediately after L-dopa administration, the nigra was stimulated electrically for 60 min. Rats were then decapitated and corpora striata ipsi- and contralateral to the stimulated side were dissected and frozen on dry ice. Stimulation parameters were: 20 μA, 1 ms in duration, at a frequency of 20 Hz. Control groups (all implanted with electrodes) included: (1) non-stimulated rats given L-dopa 60 min after pretreatment with carbidopa and killed 60 min later, (2) non-stimulated rats injected twice with saline at 60 min intervals and decapitated 60 min after the second injection, (3) rats stimulated for 60 min following saline injections and decapitated at termination of stimulation. Frozen striata were weighed and homogenized in 750 μl of 0·1 M perchloric acid.

Deproteinized aliquots were assayed for DA, dopa, DOPAC and HVA using high-performance liquid chromatography with electrochemical detection (Hefti 1979; Hefti et al 1980). The posterior parts of the brain were fixed in 10% formalin, and 40 μm frozen sections were cut serially, mounted and stained with

haematoxylin and eosin for verification of electrode placement. Only samples with appropriate location of electrode in the substantia nigra were included in the biochemical data.

Results

Unilateral electrical stimulation of the substantia nigra did not produce changes in DA levels in the ipsi–or contralateral striata compared with those in non-stimulated animals. It significantly increased both DOPAC and HVA concentrations in striata ipsilateral to the stimulated nigra (by approximately twofold) compared with those in contralateral and control striata (Fig. 1). The elevations in DA metabolites were limited to the stimulated sides and did not occur in contralateral striata (Fig. 1). These data indicate that the electrical stimulation accelerated DA synthesis and release by the activated nigrostriatal DA neurons and that its effects were restricted to the stimulated side. In electrode-implanted non-stimulated rats, L-dopa produced marked elevations in striatal DA, DOPAC and HVA levels; the increases in DA and its metabolites were similar in ipsi- and contralateral striata (Fig. 1). In L-dopa-treated rats undergoing unilateral nigral stimulation, increases in striatal DA, DOPAC and HVA concentrations in the stimulated sides did not differ significantly from those in contralateral striata and in striata of dopa-treated non-stimulated animals (Fig. 1). After L-dopa administration, dopa levels were similar in ipsi- and contralateral striata of stimulated and non-stimulated animals.

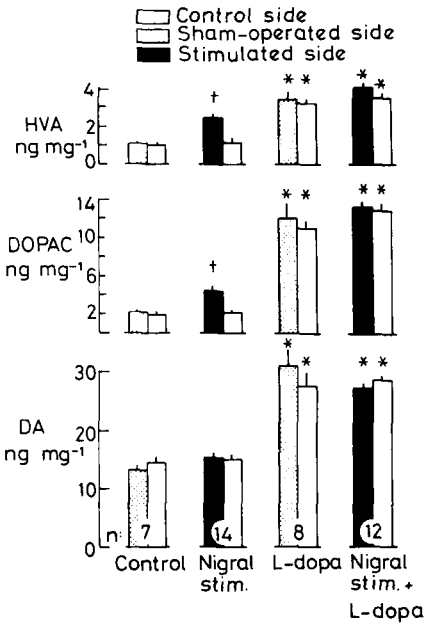

FIG. 1. Effect of combined unilateral electrical stimulation of the nigrostriatal pathway and systemic L-dopa on levels of DA, DOPAC and HVA in rat striatum. Electrodes were stereotaxically implanted into left substantia nigra. Rats were injected with either L-dopa (50 mg kg^{-1}, i.p., 60 min after carbidopa, 100 mg kg^{-1}) or saline, and stimulated (200 μA, 1 ms, 20 Hz) for 60 min. Electrode-implanted non-stimulated rats were killed 60 min after L-dopa (following carbidopa) or saline injections and served as additional controls. DA, DOPAC and HVA were measured in striata ipsi- and contralateral to implanted side. Columns represent means ± s.e.m.; number of animals in each group is given at the bottom of columns; † $P < 0.01$, compared with contralateral and control striata of rats not given L-dopa; * $P < 0{\cdot}001$, compared with striata of saline-injected rats with or without electrical stimulation (analysis of variance followed by Scheffe's test).

Discussion

Striatal levels of DOPAC and HVA are used as indices for the action potential-evoked release of DA from DA nerve terminals (Korf et al 1976; Roth et al 1976; Korf 1979). After systemic administration of L-dopa, the increases in striatal DOPAC and HVA derive predominantly from degradation of the DA formed from dopa in DA neurons but also at other loci such as endothelial cells of brain microvessels, extracerebral peripheral tissues, and even in decarboxylase-containing non-aminergic striatal neurons (Hefti & Melamed 1981a; Melamed et al 1980b,c). However, after blockade of peripheral dopa decarboxylase by carbidopa, the major fraction of the dopa-induced elevations in striatal DOPAC and HVA reflect metabolism of DA formed in nigrostriatal neurons (Melamed et al 1980b). Therefore, had the nigral stimulation increased the utilization of exogenous L-dopa by the activated DA neurons, changes should have been observed in striatal DOPAC and HVA (with or without DA alterations) to reflect enhanced synthesis and subsequent release of DA from the systemically administered precursor. However, after loading with L-dopa we found no further increases in striatal DOPAC, HVA (or DA) concentrations during electrical stimulation. Although systemic L-dopa reportedly inhibits spontaneous firing of DA neurons (Bunney et al 1973), it is unlikely that it could have counteracted the acceleration of impulse flow in the nigrostriatal projection induced by high frequency electrical stimulation. It is also unlikely that at the dose used (50 mg kg^{-1}), L-dopa could have saturated the decarboxylating capacity of striatal DA terminals. We found that after pretreatment with carbidopa, L-dopa at doses ranging from 10 to 100 mg kg^{-1}, produces linear increases in striatal DA, DOPAC and HVA levels with no plateauxing at the higher doses (Melamed et al unpublished). Our findings indicate therefore, that increased impulse flow in the nigrostriatal system is not associated with enhanced utilization of exogenous L-dopa by DA neurons.

In non-stimulated animals, DA neurons are not all in the same phase of activity but are randomly depolarized. During electrical stimulation of the nigrostriatal projection, this pattern of neuronal activity is altered, many DA neurons are simultaneously activated and their firing would to some extent, become synchronized (Korf 1979). Although this procedure artificially

accelerates impulse flow of DA neurons, it has been used extensively in the study of various biochemical and pharmacological aspects of central DA neurotransmission (Korf 1979). Both, electrical stimulation of the nigrostriatal projection (Korf et al 1976; Roth et al 1976; Korf 1979) or L-dopa administration (Hefti & Melamed 1981a,b) increase DA synthesis and release by striatal DA terminals. It is believed that when a DA neuron fires, there is a release of a specific quantity of DA molecules from its nerve-terminals. It does not seem likely that a possible effect of electrical nigral stimulation on utilization of exogenous L-dopa would not become apparent unless the basic DA stores in DA neurons are exhausted. Firstly, increases in impulse flow do not deplete neuronal DA stores. During nigrostriatal stimulation, endogenous striatal DA levels remain unaltered although synthesis and release of transmitter are enhanced (Murrin & Roth 1976; Fig. 1). In addition, during acceleration of DA neuronal firing, the newly synthesized DA molecules are the first to be released (Javoy & Glowinski 1971; Korf 1979). Thus, increases in impulse flow and coupled activation of tyrosine hydroxylase simultaneously increase synthesis of DA from endogenous tyrosine and a preferential release of the newly synthesized amine so that the net amount of DA molecules in the nerve-terminal remains constant. Consequently, if utilization of systemically administered L-dopa is dependent upon neuronal firing rates, electrical nigral stimulation should have produced increases in the release of DA molecules freshly synthesized from exogenous L-dopa irrespective of the basic amount of transmitter present within the nerve-terminals.

Several studies suggest that within DA nerve-endings, DA is stored in at least two pools (e.g. vesicular and cytoplasmic granular) and that only one of those is rapidly releaseable and regulated by neuronal firing rates (Javoy & Glowinski 1971; Korf 1979). Studies in striatal synaptosomes showed that the dopa formed in situ from tyrosine hydroxylation does not exchange freely with dopa taken up from the medium and is preferentially decarboxylated to DA (Bagchi et al 1976; Bagchi & Smith 1980). This raises the possibility that endogenous (tyrosine-derived) and exogenous dopa, and also the DA formed from either following decarboxylation, exists in two separate pools within DA neurons. The nature of the intraneuronal compartment where DA is synthesized from administered L-dopa and the mechanisms of its release from the nerve terminals are unknown. However, it is feasible that changes in DA neuronal firing affect the release of DA formed from endogenous tyrosine but not of the DA formed from exogenous L-dopa which probably occurs independently of the state of activity of DA neurons. The properties of increased impulse flow produced in the present experiment by acute electrical stimulation of the substantia nigra may differ from those of the chronic hyperactivity of DA neurons that survive after degeneration of the nigrostriatal projection. Nevertheless, our findings do not favour the hypothesis suggesting that in Parkinson's disease the remaining hyperactive DA neurons utilize more exogenous L-dopa than do neurons in normal brains (Hornykiewicz 1974). They support previous studies (Melamed et al 1980c; Hefti & Melamed 1981a) indicating that the residual DA terminals may not represent the only or major locus for conversion of systemically administered L-dopa to DA in the Parkinsonian striatum.

Supported by a grant donated by the Warschaw family in honor of Lawrence A. Harvey, by the USA–Israel Binational Science Foundation grant no. 2348, and by the Chief Scientist's Office, Ministry of Health, Israel. Esther Friedlender and Judith Levy provided technical assistance. We thank Dr F. Hefti for reviewing the manuscript.

REFERENCES

Agid, Y., Javoy, F., Glowinski, J. (1973) Nature New Biol. 245: 150–151

Bagchi, S. P., Bagchi, P., Smith, T. M. (1976) Brain Res. 187: 403–414

Bagchi, P., Smith, T. M. (1980) Ibid. 107: 591–601

Bernheimer, H., Birkmayer, W., Hornykiewicz, O., Jellinger, K., Seitelberger, F. (1973) J. Neurol. Sci. 20: 415–455

Bunney, B. S., Aghajanian, G. K., Roth, R. H. (1973) Nature (London) 245: 123–125

Hefti, F. (1979) Life Sci. 25: 775–781

Hefti, F., Melamed, E. (1981a) J. Pharmacol. Exp. Ther. 217: 189–197

Hefti, F., Melamed, E. (1981b) Brain Res. 225: 333–346

Hefti, F., Melamed. E., Wurtman, R. T. (1980) Ibid. 195: 123–137

Hornykiewicz, O. (1974) Life Sci. 15: 1249–1259

Javoy, F., Glowinski, J. (1971) J. Neurochem. 18: 1305–1311

Konig, J. H. R., Klippel, R. A. (1967) The Rat Brain: A stereotaxic Atlas of the Forebrain and the Lower Parts of the Brain Stem, Williams and Wilkins, Baltimore

Korf, J. (1979) in: Horn, A. S., Korf, J., Westernink, B. H. C. (eds) The Neurobiology of Dopamine. Academic Press, London, pp 237–253

Korf, J., Grasdijk, L., Westernink, B. H. C. (1976) J. Neurochem. 26: 579–584

Melamed, E., Hefti, F., Wurtman, R. J. (1980a) Proc. Natl. Acad. Sci. U.S.A. 77: 4305–4309

Melamed, E., Hefti, F., Wurtman, R. J. (1980b) Brain Res. 198: 214–218

Melamed, E., Hefti, F., Wurtman, R. J. (1980c) Ann. Neurol. 8: 558–563

Murrin, L. C., Roth, R. H., (1976) Mol. Pharmacol. 12: 463–475

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

Scally, M. C., Ulus, I., Wurtman, R. J. (1977) J. Neurol. Trans. 41: 1–6