The Effects of 6-Hydroxydopamine Pretreatment on the Accumulation of Dopa and Dopamine in Brain and Peripheral Organs Following L-Dopa Administration

L. D. Lytle, O. Hurko, J. A. Romero, K. Cottman, D. Leehey, and R. J. Wurtman

Laboratory of Neuroendocrine Regulation, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139, U.S.A.

Received April 18, 1972

Summary

The capacity of brain and peripheral organs to accumulate dopamine following intraperitoneal l-dopa was not impaired in rats whose catecholamine-containing nerve terminals had been destroyed by pretreatment with intracisternal or intraperitoneal 6-hydroxydopamine. These findings indicate that the uptake of exogenous l-dopa and its conversion to dopamine are not restricted to cells that normally synthesize and contain catecholamines.

Introduction

The therapeutic effect of l-dopa in the treatment of Parkinson's Disease has been attributed to its uptake within the surviving dopaminergic neurons of the nigro-striatal tract, and its decarboxylation to dopamine (DA) [1]. We have recently presented evidence that many peripheral organs have the capacity to accumulate DA following l-dopa administration; the concentration of DA present in each organ is unrelated to its basal norepinephrine (NE) and DA concentrations [2, 3]. Studies to be described in this report will show that the destruction of catecholamine-containing nerve terminals in the brain and in peripheral organs (i.e., by intracisternal or intra-
peritoneal administration of 6-hydroxydopamine [6-OHDA]) does not significantly depress the capacity of these organs to take up exogenous l-dopa and accumulate DA.

**Materials and Methods**

Thirty-two male Sprague-Dawley rats (200–250 g, Charles River Laboratories, Wilmington, Mass.) were exposed to light (Vita-Lite, Duro-Test Mfg. Co., North Bergen, N.J.) from 9 a.m. to 9 p.m. daily and given ad libitum access to Big Red Rat Chow and water. One group of 16 received an intraperitoneal injection of 6-OHDA hydrobromide (100 mg/kg of free base; Regis Chemical Co., Chicago, Ill.) followed by a second injection 48 h later. The other group received equivalent volumes (5 ml/kg) of the diluent (1 mg/ml l-ascorbic acid in 0.9% saline). Ten days after the second injection half of each group received an intraperitoneal injection of l-dopa (100 mg/kg; Hoffmann-La Roche Co., Nutley, N.J.), and the other half an equivalent volume (10 ml/kg) of the vehicle (0.05 N HCl). Thirty minutes later the animals were killed by cervical fracture and decapitated. Brain, heart, spleen, and stomach were weighed, homogenized in 0.4 N HClO₄, and centrifuged at 17,000 × g for 15 min. The dopa, DA, and NE in the supernatant fluid were separated by column chromatography. The samples were first passed over ion-exchange columns (Dowex 50-X4, 200—400 mesh, H⁺ [4]); and the dopa, DA, and NE fractions were further purified using alumina columns [5]. Dopa and NE were estimated fluorometrically by the method of von Euler and Lishajko [6]; DA was assayed by the method of Carlsson and Waldeck [7]. Overall recoveries for all three catechols varied between 50 and 55%; all data were corrected for these recoveries.

In a second experiment, two groups of 20 rats were pretreated intracisternally [8] with two doses of 6-OHDA (200 μg dissolved in 20 μl) or its vehicle, separated by 48 h. Ten days after the second injection half of the animals in each group received intraperitoneal l-dopa (200 mg/kg) and the other half its vehicle (0.05 N HCl; 20 ml/kg). The animals were killed 30 min later, and their brains removed, dissected sagittally into approximately equal halves, and frozen. Dopa, DA, and NE were determined in half of each brain, using procedures described above; serotonin (5-HT) was assayed in the other half by the method of Thompson et al. [9].

**Results**

In confirmation of previous reports [2, 3], dopa was detectable in the stomachs, hearts, and spleens of normal animals but not in their brains (Table 1). L-dopa treatment caused large increases in the dopa concentrations of all of the tissues studied. Among control animals, DA was detectable only in the brains; l-dopa administration caused all of the tissues studied to contain relatively large (i.e., 1—10 μg/g) concentrations of DA (Table 1). The tissues from control animals contained NE in concentrations ranging from 0.29 μg/g