6-HYDRXYDOPA-INDUCED STIMULATION OF ORNITHINE DECARBOXYLASE ACTIVITY IN THE DEVELOPING RAT BRAIN

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The developmental alteration in the specific activity of ornithine decarboxylase (ODC) was investigated in the brain of rats treated with 6-hydroxydopa (6-OHDOPA) at birth. The specific activity of ODC in the cerebral hemispheres, brain stem and cerebellum declined from high level on day 4 to the non-detectable level in the adult. The neonatal treatment with 6-OHDOPA (75 mg/kg, *s. c.*, 3 days) produced the rapid increase of ODC activity in the cerebral hemispheres and the brain stem in the 4- and 5-day-old rat. An injection of 6-OHDOPA appears to affect cellular activity of catecholamine neurons and glial cells in the developing brain.

Keywords—ornithine decarboxylase; 6-OHDOPA; developing rat; brain region; cellular activity

L-Ornithine decarboxylase (EC 4. 1. 1. 17; L-ornithine carboxylase, ODC), the initial and probably rate-limiting step in polyamine biosynthesis,¹⁻⁴⁾ is elevated markedly in tissues undergoing rapid growth and differentiation. The systemic injection of 6-hydroxydopa (6-OHDOPA) to neonatal rats produces a significant change of regional content of catecholamine in the developing brain, suggesting 6-OHDOPA-induced damage of nerve terminals of central catecholamine neurons.⁵⁻⁸⁾ Recently, it is demonstrated that intraventricular injection of 6-hydroxydopamine into the adult rat elicits the stimulation of ODC activity in certain brain nuclei.⁹⁾ However, little is known as to how systemic injections of 6-OHDOPA into newborn rats affect ODC activity in the regional brain (neurons or glial cells) during development. The present study was designed to determine the change in brain ODC activity in the developing rat following the neonatal treatment with 6-OHDOPA.

Wistar rats of both sexes were used. Neonatal rats from the same litter were divided into test and

control groups. The treatment with 6-OHDOPA (Nippon-Roche Research Center) were carried out by the method of Nomura and Segawa.^{10,11)} A dose of 75 mg/kg (0.1 ml/10 g of body weight) of the drug dissolved in the saline containing 0.001 N HCl or its vehicle, was first injected *s. c.* into the neonatal rat within several hr after birth and followed by the second and third injections on days 2 and 4. The weaning of pups was carried out on day 22 or 23. At the age of days 4 (5 hr after the third injection of 6-OHDOPA), 5 (24 hr after the third injection), 7, 11 and 70, animals were decapitated and brain regions were obtained by dissecting the brain into the cerebral hemispheres, the cerebellum and the brain stem. Preparation of enzyme was carried out by the method of Russell and Snyder.²⁾ An absorption of ¹⁴CO₂ was performed by the method of Schmidt-Glenewinkel *et al.*¹³⁾ After pre-incubation at 37° for 10 min, 0.1 ml of D,L-[1-¹⁴C]ornithine (2.5 μCi/ml, 59 mCi/mmol, The radiochemical Center, Amersham) was added to the enzyme preparation of 1.0 ml and the reaction mixtures were incubated for 60

FIG. 1. The Effect of Neonatal Treatment with 6-Hydroxydopa on Specific Activity of Ornithine Decarboxylase in the Cerebral Hemispheres, the Cerebellum and the Brain Stem of the Developing Rat Brain

A dose of 75 mg/kg of 6-hydroxydopa was injected s.c. into the neonates on days 0, 2 and 4. The control and the treated animals were sacrificed on day 4 (5 hr after the last injection), day 5 (24 hr after the last injection), day 7, day 11 and day 70 to measure enzyme activity. ○—○, control; ●- - -●, 6-hydroxydopa treatment. Significance, ** $p < 0.01$ vs. control.

min at 37° with continuous agitation. The reaction was stopped by injecting 0.5 ml of 2 M citric acid through the rubber caps, and the incubation continued for an additional 60 min. KOH was transferred into a scintillation vials with 10 ml of toluene scintillator, and the radioactivity was counted. A reagent blank and a blank in which enzymatic activity was destroyed by heat treatment at 80° for 30 min were used in all experiments. Protein was estimated by the method of Lowry *et al.*¹²⁾ Student's *t*-test was used for statistical analysis.

Fig. 1 shows that ODC activity was high in the neonatal brain, subsequently decreased and reached a non-detectable level in the adult brain. The activity in the cerebellum remained to be higher until later stage (day 11) than those in the cerebral hemispheres and the brain stem, suggesting a slower differentiation and maturation in the cerebellar neurons and glial cells than others. The s. c. administration of 6-OHDOPA caused a significant ($p < 0.01$) increase in the specific activity in the cerebral hemispheres and the brain stem at 5 hr and 24 hr, respectively, on day 4 and 5 after the last injection of 6-OHDOPA (Fig. 1). Since desmethylimipramine pretreatment suppressed

6-OHDOPA-induced increase of AODC activity in the certain brain nuclei of the adult rat,⁹⁾ these alteration seems to be dependent from the destruction of central catecholamine neurons. It is presumable that 6-OHDOPA-induced increase of ODC activity in the brain stem and in the cerebral hemispheres could be indirect stimulation in cell bodies of catecholamine neurons and in glial cells respectively. Work is now in progress on how neurons and glial cells are involved in ODC activity increased by 6-OHDOPA treatment.

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