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DIFFERENT ALTERATIONS IN THE DEVELOPMENT OF THE NORADRENERGIC INNERVATION OF THE CEREBELLUM AND THE BRAIN STEM PRODUCED BY NEONATAL 6-HYDROXYDOPA

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SUMMARY

The administration of 6-hydroxydopa (6-OH-DOPA) to rats during their pre- or postnatal development, produced long-term modifications in the distribution of noradrenaline (NA) within the brain. In the cerebellum, the concentration of NA was increased in adult rats exposed to the drug between the day 16 of gestation and the day of birth. When injected 3 days after birth, the drug did not modify NA levels while treatment at 20 days produced a marked depletion of cerebellar NA. The concentration of NA in the brain stem showed a different pattern of response to 6-OH-DOPA. Prenatal administration elevated NA in this region and, in contrast to the response of the cerebellum, injections in the inmediate postnatal period also elevated the transmitter content. Treatment at 20 days after birth resulted in a marked depletion of NA levels in the adult brain stem. These results demonstrate the existence of temporal differences in the responses to neonatal 6-OH-DOPA in two structures innervated by noradrenergic pathways originated in neurons of the nucleus locus coeruleus.

The neurotoxic compound 6-hydroxydopamine (6-OH-DA) and its precursor aminoacid 6-hydroxydopa (6-OH-DOPA)injected systemically to fetal or newborn rats, interferes with the normal development of central noradrenergic pathways. This results in long-term alterations in the distribution of noradrenaline (NA) within the brain, i.e., while its concentration decreases in the forebrain and the spinal cord, it is markedly elevated in the brain stem (1-12). The changes in forebrain NA have been interpreted as a result of the destruction of noradrenergic nerve terminals and those in the brain stem as reflecting an increased outgrowth of such terminals consequent to the chemical injury. This sprouting of noradrenergic axons seems to be mainly restricted to the pons, that is, to the vicinity of the cell bodies from which they originate (8,10).

Like the brain stem, the cerebellum receives its noradrenergic innervation mainly from the neurons of the locus coeruleus (13,14) and is also spatially close to the nucleus. Thus, it is possibly penetrated by the sprouts which originate in the vicinity of the locus coeruleus following 6-OH-DOPA in which case cerebellar NA would be expected to increase as it does in the brain stem. However, several studies have shown that the repeated injection of 6-OH-DA or 6-OH-DOPA after birth, produces a long-term depletion of cerebellar NA (1,6,7). Since the response to the drug depends on the developmental stage in which it is injected (11), an initial increase could be masked if the sensitivity of that particular pathway to the drug changes during the short

period of its administration.

To analyze this situation, the concentration of NA was assayed in the cerebella of adult rats which received a single injection of 6-OH-DOPA at different stages of their pre- and postnatal development and the changes were compared to those of brain stem NA. By using 6-OH-DOPA it was possible to study the response of central noradrenergic neurons to the chemical injury during their entire development because the aminoacid administered systemically crosses the placenta (7) as well as the blood-brain barrier, being decarboxylated in the brain to 6-OH-DA (for references see 15).

METHODS

Pregnant Wistar rats were injected at days 13, 16 or 17 of gestation with L-2,4,5-trihydroxyphenylalanine (6-OH-DOPA). The drug was dissolved immediately before use in 0.001 N HCl and two intravenous injections, each of 50 mg/kg, were given on the same day at an interval of 4 hr (total dose: 100 mg/kg). Animals receiving the diluent alone served as controls. Rats born on day 21 of gestation were weaned at 28 days of age. The offspring of rats receiving 6-OH-DOPA and of control rats were killed at 70 days of age.

In other experiments, neonatal rats received 6-OH-DOPA dissolved as indicated and injected subcutaneously in a volume of 100 µl. A single dose of 100 µg/g was given either on the day of birth, on day 3 or on day 20 after birth. Other animals received 50 µg/g of 6-OH-DOPA sc on the day of birth and the injection was repeated 3 times with a 48 hr interval between injections (total dose: 200 µg/g). In all cases, control animals received the diluent alone. Treated and control rats were reared together, weaned at 28 days and killed at 70 days of age.

After decapitation, the cerebellum was separated from the brain stem which was isolated from the forebrain by a coronal section between the anterior colliculi and the mamillary bodies. NA concentration in the cerebellum and the brain stem was determined fluorometrically (16) after isolation of the amine from perchloric acid extracts by cation column exchange chromatography (17). For each experimental procedure, 3-5 groups of 6-8 rats each were studied. The significance of differences between values was determined by Student's *t* test.

RESULTS

The concentration of NA in the cerebellum and the brain stem of adult rats was markedly influenced by the perinatal administration of 6-OH-DOPA as shown in Table 1. Prenatal exposure to the drug during the day 16 of gestation or later, permanently increased the content of NA in both structures. However, the changes in their NA content produced by the postnatal administration of 6-OH-DOPA although of a similar nature, followed a different temporal sequence. A single injection of the drug given on the day of birth increased the concentration of NA both in the cerebellum and the brain stem. But while four injections given on alternate days starting immediately after birth diminished the NA content of the cerebellum, they produced an increase in brain stem NA, similar to that observed in rats treated only on day 1. Cerebellar NA was not modified when a single dose of 6-OH-DOPA was injected on day 3, a treatment which produced a 63 % increase in brain stem NA levels. Finally, the concentration of NA diminished in both structures in animals receiving 6-OH-DOPA on day 20. This permanent NA depletion is the characteristic effect produced in all brain regions by the neurotoxic compounds when given to adult rats (see 15).

TABLE 1

Content of Endogenous Noradrenaline in the Cerebellum and the Brain Stem of Adult Rats Injected Perinatally with 6-OH-DOPA [a]

	CEREBELLUM		BRAIN STEM	
	ng/g	% change	ng/g	% change
Controls	232 ± 14		538 ± 17	
Gestational age when injected:				
Day 13	211 ± 11	- 9	618 ± 17	+ 6
Day 16	290 ± 7 **	+ 25	790 ± 41 **	+ 37
Day 17	499 ± 46 ***	+ 115	1049 ± 45 ***	+ 80
Postnatal age when injected:				
Day 1	464 ± 25 ***	+ 100	1044 ± 73 **	+ 79
Days 1,3,5,7	174 ± 13 **	- 25	1093 ± 40 ***	+ 88
Day 3	220 ± 9	- 5	950 ± 40 ***	+ 63
Day 20	39 ± 2 ***	- 83	396 ± 13 **	- 32

[a] Rats were injected perinatally as indicated in Methods and the content of noradrenaline in the cerebellum and the brain stem was assayed at 70 days of age.

Each value is the mean ± S.E.M. of 3-5 groups each of 6-8 rats

** $p < 0.01$; *** $p < 0.001$ significance of differences between control and treated groups

DISCUSSION

The evolution of the changes produced in cerebellar and brain stem NA by perinatal 6-OH-DOPA reveals that a reaction leading to a permanent increase of NA stores, characteristically observed in animals injected during the last days of gestation or immediately after birth, is followed by changes resulting in a permanent depletion of NA. The temporal sequence in which these responses appear differs in both structures. The sensitivity of cerebellar NA to neonatal 6-OH-DOPA changes very rapidly after birth because its content is increased by the injection on day 1, is not modified at day 3 and multiple injections on

days 1, 3, 5 and 7 not only counteract the increase which produces the first injection but decrease cerebellar NA. On the other hand, brain stem NA can be increased by 6-OH-DOPA during a longer period as shown by the fact that the elevation of NA produced by its administration on day 1 is not blocked by the multiple injections and also by the increase produced when it is injected on day 3 and even on day 9 after birth (11).

Of the two principal noradrenergic projections ascending from brain stem nuclei, the dorsal pathways originated in the locus coeruleus, seem to be more intensely affected by the neurotoxic compounds than the ventral noradrenergic pathways (18). The locus coeruleus is the site of origin of a lateral pathway that enters the cerebellum, a descending pathway that innervates the lower brain stem nuclei, overlapping with the termination of the ventral noradrenergic pathway and an ascending tract that gives rise to NA-containing terminals mainly in the cerebral cortex, the hippocampus and other forebrain regions (14). The response of these pathways originating in the locus coeruleus to the chemical injury during development is very different. The fibers of the ascending dorsal noradrenergic pathway do not seem to undergo any sprouting since forebrain NA is systematically and permanently depleted by 6-OH-DOPA in rats injected on day 16 of gestation and at all postnatal ages investigated (7,9,11, 15). On the contrary, the lateral pathway to the cerebellum and the fibers innervating the brain stem, react to the chemical injury in such a way that results in an increase of regional NA which most probably reflects the outgrowth of NA—containing terminals (8,10). This property is expressed during a very restricted period and, as has been shown, is lost during the ensuing development following different temporal patterns in the brain stem and in the cerebellum.

It has been suggested that the field of innervation of the neurons of the locus coeruleus is very extensive,i.e, a single neuron may send axonal branches to widely separated terminal areas through the different pathways (13,14,19). The varied response produced in these pathways by neonatal 6-OH-DOPA could be explained by assuming that only the axonal collaterals close to the cell bodies are able to sprout. Alternatively, if the various areas receive their innervation from different populations of neurons, peculiarities in the mode of response of these neurons, may account for the diversity observed. In any case, the maturation attained by the neuron when exposed to the drug seems to be the crucial factor in determining the nature and intensity of the response to 6-OH-DOPA because even in the same pathway the reaction varies markedly during development.

The heterogeneity of the changes in the distribution of brain NA which take place as a consequence of 6-OH-DOPA administration during the ontogeny of central noradrenergic neurons may be of help in further identifying the processes involved in the regulation of their differentiation.

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