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DIFFERENT EFFECTS OF NEONATAL VINBLASTINE ON PERIPHERAL AND CENTRAL NORADRENALINE NEURONS

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The systemic injection to newborn rats of the mitotic inhibitor vinblastine sulfate (0.25 μg/g s.c., 48 h after birth), produces marked and persistent changes in peripheral sympathetic neurons. Approximately half the neuronal population of the superior cervical ganglia was destroyed already at 16 days of age and this was accompanied by a partial but persistent depletion of noradrenaline (NA) from peripheral organs receiving a rich sympathetic nerve supply such as the heart, salivary glands and spleen. After the systemic injection of vinblastine to newborn rats, the content of NA in several brain regions remained unaltered at 45-60 days of age. To overcome the obstacle that the blood-brain barrier could represent to vinblastine penetration into the brain, the compound was injected directly into the brain of rat pups at 2 days of age (0.25-1.0 μg). When these animals were killed 45-60 days later, no changes were found in the concentration of NA in the cerebral cortex, the spinal cord or the cerebellum but NA levels were increased in the brain stem. Besides producing a partial but persistent peripheral sympathectomy, vinblastine injected either systemically or intracerebrally to newborn rats, provides a useful tool for the analysis of similarities and differences between the ontogenesis of central and peripheral NA neurons.

Vinblastine Noradrenaline neurons Neuronal development Sympathectomy Newborn rats Noradrenaline

1. Introduction

The systemic injection of the mitotic inhibitor vinblastine sulfate to adult rats, produces a marked depletion of noradrenaline (NA) in peripheral organs with a rich sympathetic innervation such as the heart (Keen and Livingston, 1970, 1971). This is accompanied by an impairment of the NA uptake capacity of sympathetic nerve terminals as well as with a decrease in the activities of tyrosine hydroxylase and dopamine-β-hydroxylase, enzymes responsible for NA synthesis. There is a latent period of 24 h before the appearance of these alterations after vinblastine injection (Keen and Livingston, 1970, 1971; Hanbauer et al., 1973, 1974). These changes are specific for neurons containing NA because vinblastine does not alter for example, peripheral cholinergic nerves or sensory neurons in dorsal root ganglia in conditions under which its effect on NA neurons is maximal (Cheney et al., 1973; Bennett et al., 1976; Johnson, 1978). Vinblastine does not modify central NA neurons probably due to its inability to cross the blood-brain barrier. Biochemical, pharmacological and structural studies have attempted to clarify the nature of the changes produced by vinblastine at the periphery. Apart from the known impairment of neurotubular function that it causes, vinblastine most probably also affects some specific function of sympathetic nerves, explaining the observed selectivity. The histochemical and ultrastructural changes produced in sympathetic nerve terminals

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by the systemic injection of vinblastine, suggest that they degenerate as they do after the neurotoxic compound 6-hydroxydopamine (6-OHDA) either by a direct action or by blockade of the axonal transport of some essential element to the terminal arborizations of the axons (Bennett et al., 1973, 1976; Gardiner et al., 1976).

When injected to neonatal rodents, vinblastine produces a marked and persistent destruction of sympathetic cell bodies in the superior cervical ganglia, an effect that can be prevented by the injection of nerve growth factor (NGF) (Menesini Chen et al., 1977; Johnson, 1978). The dose-response relation between vinblastine, impairment of ganglion cell bodies and reduction of tyrosine hydroxylase activity have been thoroughly characterized (Johnson, 1978).

Thus, vinblastine given to adult rats spares neuronal cell bodies but these are destroyed when the drug is injected neonatally. This response is similar to that observed after 6-OHDA which, when given during ontogenesis, produces a peripheral sympathectomy and also marked changes in central NA neurons (Jaim-Etcheverry and Zieher, 1971; Sachs and Jonsson, 1972). This study reports the long-term effects of vinblastine on developing peripheral neurons both at the level of their cell bodies and terminals as well as on the NA content of several brain areas after its systemic and intracerebral injection at birth. A preliminary account of these experiments has been presented (Jaim-Etcheverry and Zieher, 1981).

2. Material and methods

2.1. Treatment of animals

Newborn rats of the Wistar strain of both sexes were divided into experimental and control groups and injected 48 h after birth with 0.25 μg/g of vinblastine sulfate (Velbe, Eli Lilly & Co.). The drug was dissolved in saline before its subcutaneous injection in a volume of 0.01 ml/g body weight. Another group of animals received at 2 days of age an injection aimed at the lateral ventricle of 5 μl of saline containing 0.25, 0.5 or 1.0 μg vinblastine.

Treated and control rats that received the same volume of saline and by the same route as described, were reared together and given free access to food and water. Animals were killed at different time intervals between 16 and 60 days of age.

2.2. Tissue sampling

Rats were decapitated, the brain was exposed and the pineal gland and the olfactory tubercles were discarded. After removal of the cerebellum, the brain stem (medulla, pons, midbrain) was isolated by a coronal section between the anterior colliculi and the mamillary bodies and a section at the most caudal margin of the cerebellum. The cerebral cortex and the spinal cord were also isolated.

2.3. Noradrenaline assay

The different regions of the central nervous system as well as the superior cervical ganglia, the heart, the salivary glands and the spleen, were homogenized in 10 ml of cold 0.4 N $HClO_3$ containing 0.2% EDTA and 0.05% $Na_2S_2O_5$ for extraction of NA. The amine was isolated from the extracts by cation column exchange chromatography (Bertler et al., 1958) and the concentration of NA in the eluates was determined fluorimetrically (Häggendal, 1963). Results were not corrected for recovery which was $82.5 \pm 1.2\%$ The significance of differences between values was determined by means of Student's t-test.

3. Results

3.1. Effect of neonatal vinblastine on the development of peripheral sympathetic neurons

3.1.1. Superior cervical ganglion

The effects of the systemic administration of vinblastine to rats at 2 days of age on the subsequent development of cell bodies of peripheral sympathetic neurons was analyzed by studying the changes in the weight and NA concentration and content of superior cervical ganglia at different postnatal ages. In accordance with previous re-

ports, the long-term survival of rats injected at birth with 0.25 μg/g of vinblastine was approximately 50%. Body weight of treated rats was 25% lower than in controls at 16 and 30 days of age but no difference was observed between both groups at 60 days of age. Brain weight was similar in treated and control rats.

In normal rats, ganglion weight increased significantly between 16 and 30 days postnatally. As seen in fig. 1, after the neonatal injection of vinblastine, ganglion weight decreased in comparison to age-matched controls by 35% at 16 days and by 48% at 30 and 60 days. While the concentration of NA in the ganglion was unchanged after neonatal vinblastine at all time periods studied, NA content was significantly reduced after vinblastine injection by 28, 38 and 47% at 16, 30 and 60 days respectively. NA concentration and content in control ganglia did not vary significantly at the different ages studied.

3.1.2. Sympathetically innervated organs

The nature of the damage produced by neonatal vinblastine to the terminal portion of peripheral sympathetic neurons was determined by assaying the concentration of NA in sympathetically innervated organs of rats at different ages.

NA concentrations in the heart, the salivary glands and the spleen did not differ significantly at 16, 30 and 60 days of age and were thus taken

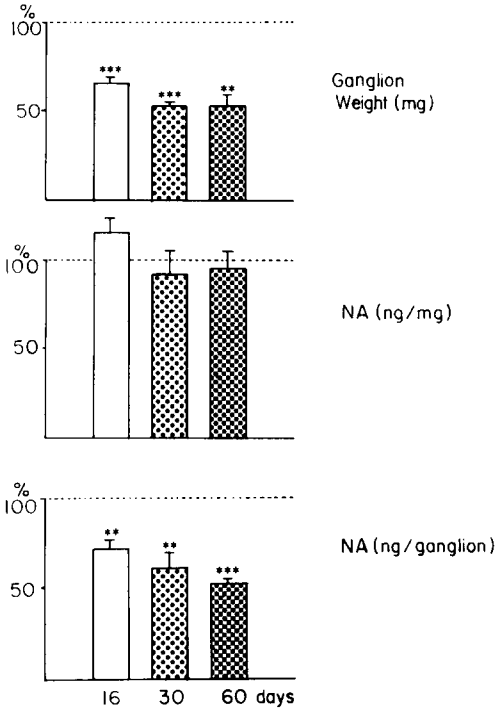

Fig. 1. Changes in the weight of the superior cervical ganglion and of its noradrenaline content and concentration in rats injected with vinblastine (0.25 μg/g s.c.) at 2 days of age and killed at 16, 30 and 60 days of age. Results are expressed as percentages of the following control values: ganglion weight (mg) 0.85 ± 0.02 (16 days), 1.21 ± 0.02 (30 days) and 1.56 ± 0.15 (60 days); NA concentration (ng/mg weight) 20 ± 1.1; NA content (ng/ganglion) 18 ± 0.8. Each value represents the mean ± S.E.M. of 4-5 groups of 4-6 rats each. **P < 0.01; ***P < 0.001 when comparing values of treated groups with corresponding controls.

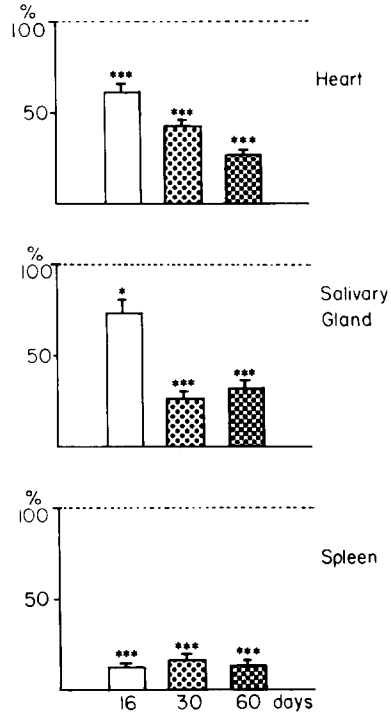

Fig. 2. Modifications in the concentration of noradrenaline of the heart, salivary gland and spleen of rats injected with vinblastine (0.25 μg/g s.c.) at 2 days of age and killed at 16, 30 and 60 days of age. Results are expressed as percentages of untreated control values. Absolute control values for NA (ng/g weight): heart 867 ± 38; salivary gland 1652 ± 81; spleen 716 ± 40. Each value represents the mean ± S.E.M. of 4-5 groups of 4-6 rats each. *P < 0.05; ***P < 0.001 when comparing values of treated groups with corresponding controls.

together for comparison although the significance of the changes produced by vinblastine was the same when results obtained at each time period were compared with values of corresponding controls.

Fig. 2 shows that vinblastine at birth markedly reduced NA levels in the heart by 39, 57 and 74% at 16, 30 and 60 days respectively. The differences between NA depletion at these ages were significant. In the salivary gland, neonatal vinblastine reduced NA by 26, 73 and 68% at 16, 30 and 60 days respectively. NA depletion at 16 days was significantly less than that observed at 30 and 60 days. NA in the spleen was reduced by more than 85% at all ages studied after the neonatal injection of vinblastine.

3.2. Effect of neonatal vinblastine on the development of central NA neurons

When vinblastine was injected systemically at 2 days of age, no changes were observed in the NA concentration of different brain regions of rats killed at 45-60 days of age (fig. 3). Since it is known that vinblastine does not cross the blood-brain barrier, this negative result was not entirely unexpected. No gross behavioral changes were observed after systemic vinblastine.

To further explore the action of the compound on central NA neurons, vinblastine was injected directly into the brain of 2 day old rats at doses of 0.25, 0.5 and 1.0 μg. No obvious behavioral or developmental changes were observed in treated animals; body weight was similar in control and treated groups at 45-60 days of age. In rats that received 1.0 μg vinblastine, an atrophic zone was apparent in the cerebral cortex at the site of injection. Long term survival in this group was of 45%. No differences were found between the weight of the brain areas studied after vinblastine and those of control rats.

As is also shown in fig. 3, intracerebral vinblastine did not modify NA levels in the cerebral cortex, spinal cord or cerebellum. In the brain

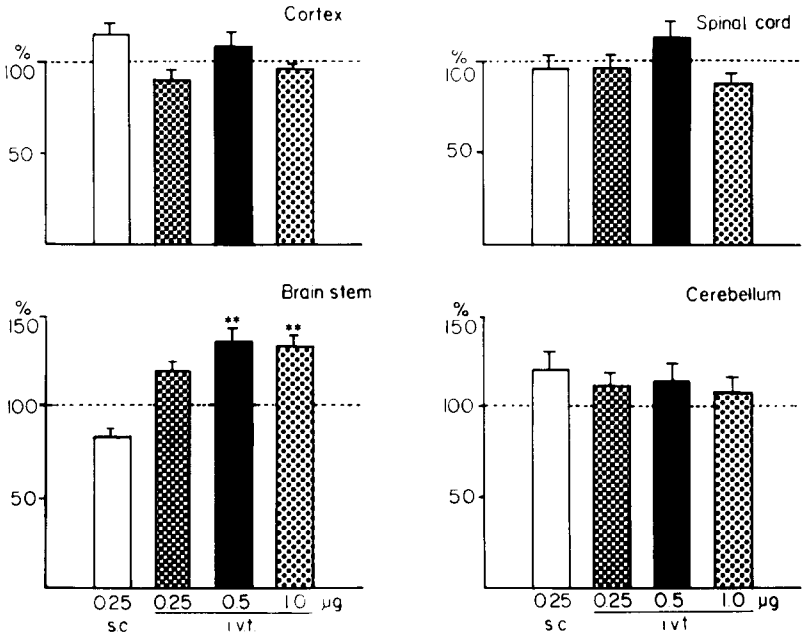

Fig. 3. Modifications in the concentration of noradrenaline of cerebral cortex, brain stem, spinal cord and cerebellum of rats injected with vinblastine (0.25 μg/g s.c. or intraventricularly with 0.25, 0.5 or 1.0 μg in 5 μl saline) at 2 days of age and killed at 45–60 days of age. Results are expressed as percentages of untreated control values. Absolute control values for NA (ng/g weight): cerebral cortex 235 ± 9.2; brain stem 584 ± 35; spinal cord 369 ± 18; cerebellum 244 ± 13. Each value represents the mean ± S.E.M. of 3-4 groups of 4-6 rats each. **P < 0.01 when comparing values of treated groups with corresponding controls.

stem however, NA levels were elevated by 37% (0.5 μg) and by 33% (1.0 μg).

4. Discussion

The injection of vinblastine to newborn rats destroys an important number of cell bodies in the superior cervical ganglia as is already noticeable at 16 days of age and becomes more marked with time. This is indicated by the reduced weight of the ganglia and by the parallel decrease in their NA content which confirm previous morphological and biochemical observations (Menesini Chen et al., 1977; Johnson, 1978). The fact that NA concentration is similar to that of control ganglia, suggests that those neurons which survive the initial lesion, develop normally since they contain a normal amount of NA.

Such an irreversible destruction of cell bodies is accompanied by a marked and sustained reduction of NA in peripheral organs that receive a rich sympathetic innervation such as the heart, salivary glands and spleen. This decrease is also evident at 16 days and is even greater at 30 and 60 days. Assays performed 3-4 months after vinblastine treatment (data not shown) confirm the irreversibility of these alterations. Thus, vinblastine given at 2 days of age, produces a partial but persistent peripheral sympathectomy similar to that triggered by the neonatal administration of the antiserum against NGF (Levi Montalcini and Angeletti, 1966) or of 6-OHDA (Angeletti, 1971; Jaim-Etcheverry and Zieher, 1971).

The systemic injection of vinblastine to adult rats does not alter their central NA neurons. When vinblastine was systemically injected to newborn rats, the content of NA in different brain regions was unchanged 45-60 days later. Since this was most probably due to the inability of the compound to enter into the brain of newborn rats as is the case in adult animals (Cheney et al., 1973), vinblastine was injected directly into the brain of rat pups. After different doses of vinblastine, the content of NA did not change in the cerebral cortex,spinal cord or cerebellum of 45-60 day old rats. In the brain stem however, NA levels increased by approximately 35% above control values after 0.5 and 1.0 μg of vinblastine injected directly into the brain.

This increase in brain stem NA is a characteristic alteration produced by neurotoxic compounds such as 6-OHDA, its precursor amino acid 6-OHDOPA or DSP 4. However, in these situations, NA decreases in the cerebral cortex and the spinal cord and increases not only in the brain stem but also in the cerebellum (for ref. see Jaim-Etcheverry and Zieher, 1977; 1980; Jonsson et al., 1982). This increase of NA in the proximal areas has been interpreted as representing the attempt of NA neurons to maintain the constancy of their arborizations (Jonsson and Sachs, 1976). It is at present difficult to reconcile this with the isolated increase of NA levels in the brain stem of adult rats treated at birth with intracerebral vinblastine. The lack of alterations of the levels of NA in the cortex or the spinal cord is also puzzling since vinblastine apparently alters directly sympathetic terminals in the periphery. Terminal areas in the brain seem to escape the toxic actions of vinblastine despite its presence in the brain as revealed by the changes observed in brain stem NA. It is at present impossible to determine if the increase in brain stem NA results from a structural change, such as a sprouting phenomenon, or from a modification in the steady state level of NA. These alterations should be better explored because they suggest a peculiar behavior of central NA neurons after vinblastine.

The response of NA neurons to neonatal vinblastine in the periphery and the brain suggests that besides producing a peripheral sympathectomy, the compound may be a useful addition to the repertoire of chemical tools for the analysis of similarities and differences between the ontogenesis of both neuronal populations.

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