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Short communication

# PERMANENT DEPLETION OF PERIPHERAL NOREPINEPHRINE IN RATS TREATED AT BIRTH WITH 6-HYDROXYDOPAMINE

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The injection of 6-hydroxydopamine to newborn rats causes the destruction of neurons in the sympathetic ganglia of the pre- and paravertebral chains and a long lasting reduction of norepinephrine in sympathetically innervated tissues and of their capacity to retain $^{3}$H-norepinephrine. A permanent partial sympathectomy is thus achieved since no recovery is observed 3 or 5 months after administration of the drug.

6-Hydroxydopamine Sympathectomy Norepinephrine
Newborn rats Adrenergic neurons

## 1. INTRODUCTION

Since the classical studies of Cannon and his associates on the autonomic system in surgically sympathectomized cats (Cannon and Rosenblueth, 1937), great interest has been shown in the development of other selective methods to produce peripheral sympathectomy. Thus, immunosympathectomy consists in the administration to newborn animals of the antiserum against nerve growth factor, a protein essential for the development of sympathetic ganglia. The antiserum destroys the sympathetic neurons and therefore a permanent denervation is achieved (Cohen, 1960; Levi-Montalcini and Booker, 1960; Sabatini, Pellegrino de Iraldi and De Robertis, 1965). A peripheral sympathectomy in adult rats is produced by the administration of 6-hydroxydopamine (6-OHDA) which results in a long lasting depletion of norepinephrine (NE) in sympathetically innervated tissues due to a selective destruction of the adrenergic nerve endings. In this case, the terminals regenerate in the weeks which follow the injection of 6-OHDA since the nerve cell bodies are not affected by the drug (Tranzer and Thoenen, 1968; Thoenen and Tranzer, 1968).

It has been recently shown that after the injection of 6-OHDA to newborn mice and rats, the neurons localized in the sympathetic ganglia are destroyed (Angeletti and Levi-Montalcini, 1970a). Therefore, similar effects are obtained by administration of 6-OHDA or of the antiserum to nerve growth factor to newborn rodents although both actions seem to be produced by entirely different mechanisms. Based on these observations it has been proposed that 6-OHDA treatment might lead to a life long chemical sympathectomy (Angeletti and Levi-Montalcini, 1970b). In this paper we report results on the long term effects of the administration of 6-OHDA to newborn rats on sympathetic nerve cell bodies and terminals. The structure and NE content of sympathetic ganglia have been studied as well as the concentration of NE and the capacity to retain exogenous amine in tissues with a rich adrenergic nerve supply.

## 2. METHODS AND MATERIAL

Wistar rats from the same litter were separated into experimental and control groups and injected within the first 10 hr after birth. 6-Hydroxydopamine (2,4,5-trihydroxyphenylethylamine hydrobromide, F. Hoffman-La Roche and Co.) was dissolved in saline immediately before use and a dose of 50 μg/g body weight in 0.1 ml was injected s.c.; the injection was repeated daily for seven days. Treated animals and normal rats injected with saline were reared together and used for experiments when aged 4 and 12 weeks, i.e.: 3 and 11 weeks after termination of 6-OHDA injections. For morphological studies the superior cervical ganglia and the celiac ganglionic complex were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer. Some ganglia were routinely embedded for light microscopy while the rest were further fixed in 1.5% osmium tetroxide, block stained with uranyl acetate and embedded in Epon. Thin sections of these ganglia were examined under the electron microscope. Groups of treated and control rats were killed 60 min after receiving 7-$^3$H-DL-norepinephrine HCl (specific activity 6.6 Ci/mmole, New England Nuclear) 10 μCi/kg i.v.; the compound was purified before use by column chromatography on alumina. Hearts were homogenized in cold 0.4 N perchloric acid and the extracts purified before use by column chromatography on Dowex 50 W-X 4 resin. $^3$H-NE was determined by liquid scintillation counting in a 1 ml aliquot of the neutralized eluate mixed with 10 ml of a toluene-Triton X 100 scintillation mixture (Patterson and Greene, 1965). Endogenous NE levels in several tissues from control and 6-OHDA treated rats were estimated after purification of perchloric acid extracts by column chromatography on Dowex 50 W-X 4. The NE was eluted with HCl and determined fluorometrically according to Häggendal (1963).

## 3. RESULTS

### 3.1. *Sympathetic nerve cell bodies*

The size of the superior cervical ganglia and of the celiac ganglionic complex was markedly reduced in the rats treated with 6-OHDA. The light microscope showed that the population of nerve cell bodies was markedly reduced although the remaining sympathetic neurons had a normal aspect. The electron microscope study carried out on these ganglia, failed to reveal any gross alteration in the morphology of the nerve cell bodies. The content of NE in control and treated ganglia is shown in table 1. The 6-OHDA treatment causes a considerable depletion of NE in the ganglia. However, if the NE present in the ganglion is expressed as μg/g weight, the concentration of the amine is practically unchanged since the reduction in the weight of the atrophied organs parallels that in the NE content. The values obtained are similar 3 and 11 weeks after interruption of the 6-OHDA treatment.

Table 1
Norepinephrine concentration in sympathetic ganglia of rats treated with 6-OHDA [a].

| Ganglia | Group | Age of rats (weeks) | Wet weight [b] | | Norepinephrine | | |
|---|---|---|---|---|---|---|---|
| | | | mg | % | μg/g | ng/ganglion | % |
| Superior cervical | Control | 4 | 1.20 ± 0.76 | | 37.19 ± 4.34 | 22.18 ± 0.99 | |
| | 6-OHDA | 4 | 0.57 ± 0.05 | −53 [c] | 33.18 ± 4.87 | 8.90 ± 0.47 | −60 [c] |
| | Control | 12 | 1.99 ± 0.34 | | 30.44 ± 2.68 | 29.14 ± 4.38 | |
| | 6-OHDA | 12 | 0.74 ± 0.10 | −63 [c] | 37.92 ± 2.26 | 13.88 ± 3.21 | −53 [c] |
| Celiac | Control | 12 | 1.80 ± 0.38 | | 25.77 ± 2.50 | 18.23 ± 4.70 | |
| | 6-OHDA | 12 | 0.67 ± 0.08 | −63 [c] | 35.75 ± 2.36 | 6.22 ± 1.36 | −66 [c] |

Results are expressed as mean values ± SE of 4–5 experiments and as percentage variations from corresponding controls.

[a] 6-OHDA (50 μg/g) injected to newborn rats for seven consecutive days.

[b] Wet weight corresponds to a pair of superior cervical ganglia and to the celiac ganglionic complex respectively.

[c] $p < 0.05$.

Table 2
Endogenous NE concentration and uptake of $^3$H-NE in hearts of rats treated with 6-OHDA [a].

| Group | Age of rats (weeks) | Norepinephrine | | $^3$H-Norepinephrine [b] | |
|---|---|---|---|---|---|
| | | μg/g | % | nCi/g | % |
| Control | 4 | 0.711 ± 0.073 | | 5.320 ± 0.689 | |
| 6-OHDA | 4 | 0.276 ± 0.025 | −61 [d] | 1.740 ± 0.321 | −67 [c] |
| Control | 12 | 0.976 ± 0.045 | | 6.382 ± 0.680 | |
| 6-OHDA | 12 | 0.462 ± 0.038 | −53 [d] | 2.864 ± 0.180 | −55 [c] |

Results are expressed as mean values ± SE of 4–5 experiments and as percentage variation from corresponding controls.

[a] 6-OHDA (50 μg/g) was injected to newborn rats during seven consecutive days.
[b] Rats received 10 μCi/kg DL-7 $^3$H-norepinephrine i.v. 60 min before being killed.
[c] $p < 0.01$.
[d] $p < 0.001$.

Table 3
Norepinephrine concentration in salivary glands, spleen and vas deferens of rats treated with 6-OHDA [a].

| Group | Age of rats (weeks) | Norepinephrine | | | | |
|---|---|---|---|---|---|---|
| | | Salivary glands | | Spleen | | Vas deferens |
| | | μg/g | % | μg/g | % | μg/g |
| Controls | 4 | 1.364 ± 0.152 | | 0.395 ± 0.053 | | 15.18 ± 0.25 |
| 6-OHDA | 4 | 0.561 ± 0.083 | −59 [b] | 0.077 ± 0.009 | −80 [b] | 18.21 ± 1.32 |
| Control | 12 | 1.321 ± 0.050 | | 0.323 ± 0.061 | | 16.71 ± 0.62 |
| 6-OHDA | 12 | 0.600 ± 0.076 | −55 [b] | 0.052 ± 0.011 | −84 [b] | 16.02 ± 1.76 |

Results are expressed as mean values ± SE of 4–5 experiments and as percentage variation from corresponding controls.

[a] 6-OHDA (50 μg/g) was injected to newborn rats for seven consecutive days.
[b] $p < 0.001$.

### 3.2. *Sympathetic nerve terminals*

The content of endogenous NE and the retention of $^3$H-NE were markedly decreased in the hearts of rats examined 3 and 11 weeks after interruption of 6-OHDA administration (table 2). The comparison of values obtained 11 weeks after treatment with those corresponding to 3 weeks, shows an increase of approximately 10% in endogenous NE as well as in the retention of $^3$H-amine. A similar increase is observed in hearts from normal rats. In table 3 it may be seen that 6-OHDA greatly reduced the NE content of the salivary glands and spleen. The differences between the values obtained at 3 and 11 weeks after interruption of treatment are not significant. In contrast, the NE content of the vas deferens is unaffected by 6-OHDA administration.

Since NE levels in the central nervous system (hypothalamus, brain stem and hemispheres) and in the adrenal glands were unchanged 3 weeks after 6-OHDA treatment, they were not investigated further.

In rats examined 5 months after 6-OHDA administration, the depletion of endogenous NE in the ganglia, heart, salivary glands and spleen was similar to that found in these tissues 11 weeks after interruption of the treatment.

## 4. DISCUSSION

The reduction in the volume of sympathetic ganglia of the para- and prevertebral chains observed 3 and 11 weeks after the injection of 6-OHDA to newborn rats during seven consecutive days, was found to be due to a decrease in the number of sympathetic nerve cell bodies. This irreversible loss which confirms previous observations of Angeletti and Levi-Montalcini (1970a,b), is also reflected in a marked reduction of NE content in the ganglia. The neurons which remain seem to have escaped the cytotoxic effect of 6-OHDA since they do not show ultrastructural alterations and retain the capacity of storing a normal amount of NE.

The NE content of peripheral adrenergic nerve terminals of the heart, salivary glands and spleen is also greatly reduced after injection of 6-OHDA and the retention of $^{3}$H-NE in the heart is reduced to approximately the same extent as the content of endogenous amine. While in the salivary glands and in the spleen there is no recovery in the content of NE between both periods examined, in the heart the endogenous NE and the amount of $^{3}$H-NE retained 11 weeks after treatment show an increase of approximately 10% compared with values obtained at 3 weeks. This recovery may be correlated with the fact that in hearts from normal rats there is also an increase in endogenous NE concentration between 4 and 12 weeks of age. This is in accordance with the finding that the outgrowth of nerves in atria surpasses the growth of extraneuronal tissue and results in an increased density of nerves and in an increased uptake per gram with age (Sachs et al., 1970).

The degree of denervation attained in organs innervated by neurons located in pre- and paravertebral ganglia is almost the same. In contrast, the peripherally located adrenergic ganglia, e.g. the neurons supplying the vas deferens, seem to be unaffected by the drug as are the central nervous structures and the adrenal gland. The difference in susceptibility of peripheral adrenergic neurons to 6-OHDA, which are also observed with the antiserum (Levi-Montalcini and Angeletti, 1966), are probably due to the fact that the cytotoxic agents act on neurons which have attained different stages of differentiation. This circumstance might also explain the variation in NE depletion between the tissues studied and the species differences observed, mice being more susceptible than rats to the effect of 6-OHDA (Angeletti and Levi-Montalcini, 1970b). The lack of effect of 6-OHDA on central monoaminergic neurons probably reflects the existence of a well developed blood-brain barrier for circulating monoamines in newborn rats.

When 6-OHDA is injected to adult rats, the peripheral adrenergic nerve terminals are selectively destroyed, NE decreases in the sympathetically innervated tissues and their capacity to retain exogenous NE is diminished. Since the cell bodies of sympathetic neurons are not affected by the drug, the terminals regenerate and the endogenous NE as well as the capacity to retain $^{3}$H-NE are completely restored approximately 8 weeks after the injection (Thoenen and Tranzer, 1968; Jonsson and Sachs, 1970). In contrast, when 6-OHDA is administered to newborn rats in which adrenergic nerve cell bodies are susceptible to the effect of the drug, they undergo degeneration and disappear from the ganglia (Angeletti and Levi-Montalcini, 1970b). Some cells escape this cytotoxic action and account for the NE which remains in the ganglia and for the residual innervation of peripheral organs. In these tissues there is a decrease in endogenous NE as well as in the retention of exogenous amine. However, no recovery is possible in this case since the entire adrenergic neuron is destroyed. These differences in the response to 6-OHDA of sympathetic neurons in newborn or adult animals, might reflect the ability of the adrenergic nerve cell body to take up exogenous amines during the early stages of development, a capacity which seems to be lost in the course of the ensuing differentiation process.

The injection of 6-OHDA to newborn rats constitutes a potentially useful tool for achieving a permanent sympathetic denervation of experimental animals. The amount of sympathetic nerve cell bodies destroyed and therefore the degree of peripheral denervation achieved, could be further increased by properly adjusting the dosage or the administration schedule of 6-OHDA.

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