Growth Inhibition of Sympathetic Cells by Some Adrenergic Blocking Agents

(6-hydroxydopamine/mice and rats/immunosympathectomy/chemical sympathectomy/superior cervical ganglion)

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ABSTRACT Treatment of newborn mice and rats with the adrenergic blocking agents, guanethidine and bretylium tosylate, results in massive destruction of immature sympathetic nerve cells. A growth inhibition of the same cells is caused by reserpine. Similarities and differences between the effects elicited by these three ganglion blocking agents and a dopamine analog, 6-hydroxydopamine, are discussed.

We have shown that the developing sympathetic nervous system can be permanently destroyed either by an antiserum to the nerve growth factor (immunosympathectomy) or by 6-hydroxydopamine, a toxic analog of dopamine (chemical sympathectomy) (1-3). While the site and mechanism of action of the cytotoxic effect of the antiserum to the nerve growth factor are still not fully elucidated, the toxic effect of 6-hydroxydopamine has been related to its chemical and pharmacological properties (4). These results suggested the possibility that other drugs that are also selectively taken up by nerve end terminals may likewise interfere with the normal development of adrenergic sympathetic nerve cells. The results to be reported below confirm this hypothesis.

METHODS

Newborn mice and rats were used in these experiments. Littermates were divided in two groups; one was injected with the test drug and the other with saline. Test drugs and saline were injected subcutaneously once a day or every other day for various lengths of time. The animals were sacrificed at time intervals between the end of the first and the fourth week and the sympathetic ganglia were dissected out under a stereomicroscope, fixed, stained with toluidine blue, mounted *in toto* or sectioned at 12 μ m, and used for histological studies. Sensory ganglia, parasympathetic ganglia, adrenal medulla, brain, and spinal cord were also dissected out and examined. A number of superior cervical ganglia of treated and control animals were fixed for 2 hr in 3% glutaraldehyde in phosphate buffer (0.1 M, pH 7.4) with added 0.01 M CaCl_2 . The specimens were washed for 2 hr in the same buffer, postfixed in osmic hydroxide (1.33%) for 2 hr, and finally embedded in Epon 8/12 for electronmicroscopic examination. The drugs used in these experiments were: bretylium tosylate (Darentyn-Wellcome Lab.), guanethidine (Ismelin-Ciba), and reserpine (Serpasil-Ciba). The dosage and time schedule for each treatment are given in the text. For each series, 15 experimental and 15 control animals were used.

Abbreviation: 6-OHDA, 6-hydroxydopamine.

RESULTS

Daily injection of bretylium tosylate (100 μ g/g of body weight) in newborn mice and rats during the first week of life results in considerable decrease in the size of sympathetic chain ganglia. At the histological examination, a large number of neurons exhibited extensive cytoplasmic vacuolization; many nerve cells were in an advanced stage of degeneration. Continuation of the treatment for 2-3 weeks resulted in an even more severe volume reduction of the ganglia and a

FIGS. 1-4. Cross sections of superior cervical ganglia of control (Fig. 1) and bretylium tosylate-treated rats (Fig. 2), killed at 10 days of age. Figs. 3 and 4 show cross sections of superior cervical ganglia of control (Fig. 3) and guanethidine-treated rats (Fig. 4) killed at 15 days of age.

decrease in the number of sympathetic neurons (Figs. 1 and 2). At the inspection under the electron microscope, a most unusual alteration was observed. All mitochondria were swollen to a tremendous size. The cristae, extending from the inner membrane, were totally disrupted, floating freely in the large and empty cavity (Figs. 5 and 6). In contrast, the other cell organelles, such as the Golgi complex and the endoplasmic reticulum, appeared normal. Likewise the nucleus showed no lesions at this early stage of treatment. The altered sympathetic neurons were surrounded by normal satellite and glial cells. At the end of the first month, sympathetic ganglia of animals treated with bretylium tosylate were severely reduced in size (Fig. 9). At inspection under the stereomicroscope, sensory and parasympathetic ganglia, as well as adrenal medulla, brain, and spinal cord of experimental animals, compared in size to those of controls. Experimental mice and rats inspected throughout the period of treatment until the end of the first month were as vital as controls and no toxic side effects were apparent.

Guanethidine was injected in newborn mice and rats at a dose of 15 $\mu\text{g/g}$ of body weight, once a day for a 10-day period. At microscopic inspection, sympathetic chain ganglia of experimental animals were not greatly reduced in size at the end of the first week of treatment; the reduction became more apparent toward the end of the second and third weeks. Histological examination of sections of superior cervical ganglia in experimental and control mice and rats, performed at 10 days and at the end of the second week (Figs. 3 and 4), showed a most impressive loss of ganglionic nerve cells in

FIG. 5. Electronmicrograph from section of superior cervical ganglion of 12-day-old rat injected since birth with bretylium tosylate. Swollen mitochondria (*m*) fill part of the cytoplasmic area. Other cell organelles apparently normal. (*n*) nucleus. Altered adrenergic neuron surrounded by normal satellite cells (*s*).

FIG. 6. Electronmicrograph showing swollen mitochondria (*m*) at higher magnification than in Fig. 5. (*G*) Golgi complex, (*e*) endoplasmic reticulum.

ganglia of treated animals and a marked increase of fibroblastic cell types between the end of the first and second week of treatment. In subsequent stages, sympathetic chain ganglia of experimental mice and rats were reduced to exceedingly small nodules. The volume reduction of superior cervical ganglia in one-month-old mice is of the same range as that obtained with injection of 6-hydroxydopamine (Figs. 7 and 8). As in the case of bretylium tosylate treatment, spinal and parasympathetic ganglia and other organs of guanethidine-injected mice were comparable to those of controls. The animals appeared to be as well developed and vital as controls.

A less-pronounced effect was obtained by injection of reserpine in newborn mice and rats. The sedative effect of the drug prevented the injection of large doses of this pharmacological agent for prolonged time periods. The animals became, in fact, sleepy to the point of dying of starvation. When reserpine was injected at lower doses, not exceeding 0.5 $\mu\text{g/g}$ of body weight every other day, a slight decrease in size of sympathetic chain ganglia was apparent, as shown in Fig. 10. At the histological examination, a significant percentage of the sympathetic nerve cell population in treated animals showed less-intense basophilia as compared to that of controls. A comparison of the structure of the ganglion blocking agents, bretylium tosylate, guanethidine, and 6-hydroxydopamine is given in Fig. 11.

DISCUSSION

Injection of the adrenolytic agent guanethidine in newborn mice and rats during the first days after birth resulted in a

FIGS. 7-10. Whole mounts of superior cervical ganglia of control (C) and experimental (X) littermate mice injected with 6-hydroxydopamine (Fig. 7), guanethidine (Fig. 8), bretylium tosylate (Fig. 9), and reserpine (Fig. 10). Animals were killed after 1 month (Figs. 7-8) and 15 days (Figs. 9-10).

massive destruction of sympathetic nerve cells. Sympathetic ganglia were reduced to exceedingly small nodules. The reduction of the size of sympathetic ganglia was of the same magnitude and severity as that called forth by immunosympathectomy or treatment with 6-hydroxydopamine (3).

Another adrenolytic agent, bretylium tosylate, also produced degenerative effects in the immature adrenergic neurons, characterized by a dramatic swelling of mitochondria. At variance with the effect described in animals treated with the antibody to the nerve growth factor or with the dopamine analog 6-hydroxydopamine, the lesion at the ultrastructural level was localized in the mitochondria, as indicated in the *Results*. Other cell organelles appeared normal.

Marked decrease in size of the same ganglia was produced by another adrenolytic agent, reserpine. The effects were less marked than with guanethidine and bretylium tosylate. Since reserpine produces a sedative effect, this lesser effect may be due to the impossibility of injection of the drug at its effective concentration.

A common feature of all these ganglion blocking agents, including 6-hydroxydopamine, is their selective accumulation in the sympathetic neurons. They gain access to the ganglionic cells through the end terminals, thus blocking adrenergic transmission. Studies on the pharmacological action of bretylium tosylate by Boura *et al.* in 1960 (5) gave the first evidence that this agent selectively impairs the function of adrenergic neurons without affecting other sections of the nervous system. The same authors showed that ^{14}C -labeled bretylium is selectively taken up by sympathetic postganglionic nerves. 18 Hr after injection of this drug (10 mg/kg

BRETYLIUM TOSYLATE

GUANETHIDINE

6-HYDROXYDOPAMINE

FIG. 11. Comparison of the structure of three ganglion blocking agents.

of body weight), its concentration in the postganglionic cervical sympathetic nerve reaches 700 nM/g, whereas in the vagus and phrenic nerves it is only 40 nM/g.

Guanethidine and bretylium tosylate also selectively bind with sites specifically related to noradrenaline storage. Evidence has been obtained that the blockade of adrenergic neurons and the loss of noradrenaline from nerve endings result from the primary action of these ganglion blocking agents (6, 7). Both drugs, as shown in these studies, cause irreversible damage to immature sympathetic nerve cells in newborn mice and rats. Other adrenergic ganglia, such as the ganglionic complexes innervating the sex glands, have yet to be investigated.

It is conceivable that other drugs interfering with the normal uptake and storage of the neurotransmitter may also call forth retrograde degeneration of immature sympathetic nerve cells. Studies to explore this possibility are now in progress in our laboratories.

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