Modulatory Effect of the Sympathetic Nervous System on Neuroblastoma Tumor Growth

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ABSTRACT

Growth of C-1300 neuroblastoma was markedly suppressed in mice chemically sympathectomized at birth with 6-hydroxydopamine. Growth of A-10 adenocarcinoma was also somewhat reduced.

In newborn mice pretreated with nerve growth factor to induce sympathetic nervous system neuronal hypertrophy, neuronal maturation, and peripheral hyperinnervation, the growth of neuroblastoma was augmented.

INTRODUCTION

Catecholaminergic neurons are sensitive to the neurotoxic action of 6-OH-DA. The mechanism of action of the agent is imperfectly understood (2). The specificity of drug action is thought to be related to uptake and accumulation of 6-OH-DA by a transport mechanism found in catecholaminergic neurons (11). The cytotoxicity of the drug is believed to be linked to its ease of autooxidation, which leads to intracellular accumulation of toxic products such as hydrogen peroxide, superoxide radical, hydroxyl radical, and possibly quinones (7).

When 6-OH-DA is given to adult animals, sympathetic nerve endings are destroyed to produce an "axotomy," but the block of sympathetic function is reversible over time when drug administration is stopped. When 6-OH-DA is administered to newborn animals, sympathetic neurons die, and an irreversible sympathectomy results (6, 8).

NGF is a basic polypeptide hormone the active molecule of which is a dimer comprised of 2 identical subunits each with a molecular weight of 13,256. NGF is essential for the growth, development, and maintenance of the SNS throughout life (1). Administration of NGF to newborn mice leads to a gross hypertrophy of the SNS, which is maintained for some time after hormone treatment is stopped (9).

Many tumors are now known to synthesize and secrete NGF, and, in fact, NGF was first discovered in tumors into which sympathetic nerves were growing (3, 4, 8, 10, 12-14). Were a SNS innervation beneficial to a tumor, its growth might be expected to be favored in an animal with a previously hypertrophied and primed SNS and conversely to be retarded in a sympathectomized animal.

Recently, we reported that growth of NB was slowed in mice axotomized as adults by pretreatment with 6-OH-DA, but the growth of A-10 was not affected (5). Drug was stopped before tumors were transplanted so that beginning recovery of SNS function during tumor growth could have lessened the magnitude of the effect observed.

Accordingly, we have studied NB growth in mice permanently sympathectomized by treatment with 6-OH-DA during the newborn period. In addition, we have induced SNS hypertrophy in newborn mice by pretreatment with NGF and have studied NB growth in them.

MATERIALS AND METHODS

NB was originally obtained from The Jackson Laboratory, Bar Harbor, Maine. A-10 was obtained as a gift from Dr. K. McCully, Department of Pathology, Massachusetts General Hospital, Boston, Mass. Both tumors are carried by serial s.c. transplant, NB in A/J mice and A-10 in A/He mice. Tumor cell suspensions were prepared by gentle homogenization of s.c. tumors. Tumor cell viability was determined with trypan blue.

Newborn A/J mice were given i.p. injections daily for 10 days of 6-OH-DA (6-OH-DA hydrobromide; Sigma Chemical Co., St. Louis, Mo.) in a dose of 100 µg/g of body weight. The solution used contained 6-OH-DA, 10 mg/ml, and ascorbic acid (as an antioxidant), 0.1 mg/ml, in 0.9% NaCl solution. Controls received 0.9% NaCl-ascorbic acid solution. At weaning (21 days) 10^6 dispersed viable NB or A-10 cells were injected in the flank as described earlier (5). Tumors were excised and weighed 10 days later.

NGF, 2.5S (a gift from Dr. M. Young), was prepared by modifications (14) of the technique of Bocchini and Angeletti. Bioactivity was determined by the chick sensory ganglion bioassay technique (kindly performed for us by Dr. R. Murphy). For the preparations used 1 biological unit approximated 10 ng. Newborn mice received NGF, 2 µg/g of body weight, daily by i.p. injection for 10 days. Controls received daily i.p. 0.9% NaCl solution. Two days after the final NGF injection, the mice were given s.c. injections of 10^6 viable NB cells as described above. The tumors were weighed 10 days later.

Statistical analyses of tumor weights were done with Student's t test.

RESULTS

NB tumors were, on the average (Chart 1), 7 times smaller in sympathectomized than in control mice, a significant difference (p < 0.001). A-10 tumors were 40% smaller in sympathectomized mice than they were in controls (p < 0.01). In NGF-pretreated mice, tumors were 3 times larger than they were in controls of like age (p < 0.05).

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3 The abbreviations used are: 6-OH-DA, 6-hydroxydopamine; NGF, nerve growth factor; SNS, sympathetic nervous system, NB, mouse C-1300 neuroblastoma; A-10, mouse A-10 breast adenocarcinoma.

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6-OH-DA-treated mice showed bilateral ptosis, evidence of successful sympathectomy. They appeared healthy but were smaller than controls. When tumor weights in individual mice were calculated as a percentage of body weight, mean values for 6-OH-DA-treated mice were 0.41% for NB versus 1.85% for controls, 5.1% for NGF-treated mice inoculated with NB versus 1.5% for controls, and 4.9% for the A-10 tumor in 6-OH-DA-treated mice versus 4.9% for controls.

DISCUSSION

Two major findings emerge from the data presented: (a) NB growth is drastically suppressed in mice chemically sympathectomized at birth, and the growth of A-10 adenocarcinoma is also somewhat reduced; and (b) NB growth rate is augmented in mice with a hypertrophied SNS. Taken together the 2 sets of data point to a modulating role for the SNS in NB growth and possibly in that of the A-10 tumor.

No ready explanation for these results is at hand. Conceivably, trophic factors released from sympathetic endings influence the tumor or its vascular supply. Were this the case, elaboration of NGF by tumors and ingrowth into them of sympathetic axons might find a logical basis.

The chemically sympathectomized mice in these experiments were smaller than their littermate controls. Similar results have been found by others; modest but general growth failure may follow early sympathetic denervation. Growth failure induced by other means (for example, by malnutrition) may slow tumor growth, and it is possible that "growth failure" contributed to the results obtained. Indeed, when tumor size was calculated as a percentage of body size the difference in growth of A-10 tumor between 6-OH-DA-treated mice and controls disappeared. It seems improbable that growth failure alone can account for the overall effect on NB growth, since such a mechanism will not explain the augmented NB growth observed in hyperinnervated mice; even when tumor size is calculated as a percentage of body weight, a 4-fold reduction in NB growth rate remained in 6-OH-DA-treated mice.

In previously published studies we observed a 50% reduction in the rate of NB growth in mice axotomized as adults with 6-OH-DA, but the A-10 adenocarcinoma grew normally in such mice. Here we find a 7-fold reduction in NB growth in sympathectomized mice, while A-10 tumor growth rate is reduced by 40%. The more striking results obtained in the present experiments may simply reflect the totality and permanency of the sympathetic denervation achieved. Whatever the explanation, the 2 sets of experiments do complement and reinforce each other. We would argue that NB is particularly susceptible to influences exerted by the SNS. Whether this susceptibility relates to the origin of NB from sympathetic tissue cannot be resolved at present.

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REFERENCES


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