LEVEL OF THE NERVE GROWTH FACTOR ACTIVITY IN THE SUBMAXILLARY GLANDS OF GENETICALLY DYSTROPHIC MOUSE (C57BL/6J)*

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SUMMARY

To examine the relationship between NGF activity and neurologic disorders, the level of NGF activity was studied in the submaxillary glands of both normal and dystrophic mice. The level of NGF activity in the mice was remarkably lower than that observed in normal mice. The data suggest that NGF activity may have some relation to the cause of the disease.

Muscular dystrophy is a term applied to a large number of the inherited diseases of skeletal muscle. The diseases occur not only in man, but also in a variety of other animals. A stock of mice segregating for animals suffering from a form of hereditary, progressive muscular dystrophy has been established from a mutation which occured in the breeding stocks of inbred strain 129 mice at the Jackson Memorial Laboratory (1).

The disease is characterized by progressive muscular weakness and gross atrophy of muscles. It is recognized by three weeks of age and in some case, as early as two weeks. Inheritance has been shown to follow the pattern of an autosomal recessive gene.

Until recently, muscular dystrophy was considered to result from a genetically determined defect within the muscle fibers themselves. Evidence is now accumulating which suggests that dystrophic individuals have an abnormality within the nervous system which may itself be largely responsible for the muscular disorder, namely an abnormal trophic influence on the affected muscles (2-6). The NGF has been shown to have pronounced stimulating effects on the developing sympathetic nervous system (7). In the course of our study on the relationship between the NGF activity and

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Abbreviation: NGF, nerve growth factor.

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neurologic disorders, we determined level of the NGF activity in the submaxillary glands of genetically dystrophic mice.

We report here our discovery of altered levels of NGF activity in submaxillary glands of homozygous dystrophic mice (C57BL/6J strain:dy/dy).

MATERIALS AND METHODS

Dystrophic Mice: The mice used were genetically dystrophic mice designated C57BL/6J strain of fifty one to fifty eight day old obtained from Central Institute for Experimental Animals, Kawasaki, Japan.

Bioassay of NGF Activity: A pair of submaxillary glands from each mouse were homogenized in a cold phosphate buffered saline (10% w/v). The homogenate was centrifuged at 4,000 rpm for 10 min, and the supernatant fluid was measured for NGF activity. The assay for NGF activity was made using dorsal root ganglia from 8-9 day chick embryos explanted in hanging drop tissue cultures described by Varon *et al.* (8). Mouse 7S NGF was used as a standard in the bioassay (8). Protein concentrations were determined by the method of Lowry *et al.* (9).

RESULTS

NGF Activity in the Submaxillary Gland: The body weight of homozygous dystrophic mouse (dy/dy) was about half that of heterozygous dystrophic mouse (dy/+ or +/+). The weight of a pair of submaxillary glands was almost proportional to body weight (0.25-0.35% w/w). Table I shows the NGF activity in submaxillary glands of homozygous (dy/dy) and heterozygous (dy/+, +/+) dystrophic mice of the C57BL/6J strain. The submaxillary gland supernatants of heterozygous dystrophic male mice elicited the standard nerve fiber outgrowth (+4 response) at a concentration from 2 μg to 7 μg protein/ml, but those of homozygous dystrophic mice required from 60 μg to over 9,000 μg protein/ml for the nerve fiber outgrowth (+4 response).

As shown in Table I, remarkable differences between heterozygous and homozygous dystrophic mice were observed. The level of the NGF activity in the submaxillary glands of male homozygous dystrophic mice was more than two order less than that of male heterozygous dystrophic mice. Level of the NGF activity in the submaxillary glands of female homozygous dystrophic mice was too low to detect the NGF activity by the concentration of about 9,000 μg protein/ml. Although, in general, level of NGF activity of female mice is lower than that of male mice, their differences are about one order (11). At present, this considerable difference between the activities in the submaxillary glands of heterozygous dystrophic male and female mice is obscure. The male heterozygous dystrophic mice were showed almost a constant specific NGF activity (2-7 μg protein/Biological Unit) amoung the individuals. However, the

Table I NGF Activity in Submaxillary Glands of Dystrophic Mice

	Body Weight (g)	Protein Concentration Required to Elicit +4 Response (μg Protein/ml)	Biological Unit /mg Protein	Significance
male heterozygous dystrophic mice (dy/+ or +/+)	20.0	7	140	$P < 0.01$
	20.0	7	140	
	20.5	2	500	
	23.0	7	140	
	21.0	7	140	
male homozygous dystrophic mice (dy/dy)	9.0	500	2	
	9.0	9000(+3)[a]	0.1	
	10.5	9000	0.1	
	10.0	500	2	
	12.0	63	16	
female heterozygous dystrophic mice (dy/+ or +/+)	15.5	9000	0.1	
	15.5	3000	0.3	
	18.5	500	2	
	17.0	3000	0.3	
	17.5	3000	0.3	
female homozygous dystrophic mice (dy/dy)	9.5	9000(±)[b]		
	9.0	9000(±)[b]		
	8.5	9000(±)[b]		
	5.5	-[c]		

a) Nerve fiber outgrowth was +3 response at 9000 μg/ml.
b) Nerve fiber outgrowth was ± response at 9000 μg/ml.
c) Nerve fiber outgrowth was not observed at protein concentration by 9000 μg/ml.

male homozygous dystrophic mice showed a wide distribution of the values (60 μg protein over 9,000 μg protein/Biological Unit), which may reflect the individual degrees of disease.

DISCUSSION

It is known that level of the NGF activity in submaxillary glands of mouse is remarkably high, compared to other animals. The content of NGF activity in mouse submaxillary glands is reported to be regulated by testosterone (10). Also, the adult male mouse contains higher amounts of NGF than female and immature mouse (11).

NGF is widely distributed in the sympathetic chain ganglia of vertebrates and seems to be essential for differentiation and development of embryonic sympathetic nervous system and to maintain that function after birth. A trace of NGF activity is also found in homogenates of striated muscle of the adult mouse as found in many sympathetic innervated peripheral organs (11). Hendry and Iversen (12) reported that the removal of the submaxillary glands in adult mice was followed by a decrease in the

tissue and plasma levels of NGF. These changes in NGF levels were accompanied by a decreased activity of tyrosine hydroxylase both in the superior cervical and the stellate ganglia (13). The reduction in tyrosine hydroxylase activity paralleled the fall in the tissue levels of NGF suggesting a causal relationship between these effects.

After removal of submaxillary gland an initial decrease in body weight was found (13). Ten days after removal mice had lost 12% of their body weight and regained only 4% by thirty days. The decrease in body weight is the one of properties generally found in muscular dystrophic mice, and suggests the close relationship between the function of submaxillary gland, especially the synthesis of NGF, and muscular dystrophy of mice. Also, it is considered that an inhibitor which affects the NGF activity may appear in the submaxillary gland of dystrophic mouse.

On the other hand, Kure and Okinaka (14) reported a muscular dystrophy-like disease caused in the infant dog by the removal of superior cervical ganglia. These results suggest that muscular dystrophy is remarkably concerned with the innervation of sympathetic nervous system. They found that morphological hypertrophy and /or atrophy of muscle fiber was elicited in the infant dog with removal of superior cervical ganglia, and these results suggest that the degree of disorder of muscles is paralleled to the degree of autonomic innervation.

We have now demonstrated a deficiency in NGF activity in the submaxillary glands of the homozygous dystrophic mice; and thus a disfunction of the sympathetic ganglia and the innervation of the sympathetic nerves to skeletal muscles may be related to the development of the disease.

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