Nerve growth factor selectively stimulates the degradation of chondromucoprotein in the embryonic cartilage in vitro

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Summary. Nerve growth factor specifically stimulates the degradation of chondromucoprotein in the chick embryonic cartilage cultivated in vitro, with little effect on chondromucoprotein, RNA, DNA and protein synthesis.

Nerve growth factor (NGF), a protein necessary for the development and maintenance of sensory and sympathetic neurons[2], was first discovered in mouse sarcome 180 implanted in the chick embryo[3]. Subsequent studies showed that NGF is most abundant in male mouse submaxillary gland[4]. NGF is probably made by submaxillary gland and circulates in the serum to be stored in other tissues. In fact, a small amount of NGF was also found in many animal tissues including the heart, the spleen, the kidney, the skeletal muscle[5] and the granulation tissue[6]. Therefore, NGF may have a fundamental effect on the cell metabolism, even if it is in the extraneuronal tissues. In this paper, I have tried to investigate the effect of NGF on the in vitro synthesis and degradation of chondromucoprotein in the chick embryonic limb bone.

Materials and methods. The femora from 9 day chick embryo, whose entire mass was composed of cartilage with no sign of bone marrow formation, were cultivated in a chemically defined medium by the roller tube method[7]. Pair-mate culture was employed. One femur from the chick embryo served as control and the other femur was cultivated in the medium containing NGF. The medium used for cultivation was a chemically defined medium BGJb-HW2, which was confirmed to permit much better chondrogenesis by supplementing the BGJb-HW[8] with glycine (2 mM), serine (1 mM), proline (1 mM), sodium ascorbate (50 µg/ml) and ferric chloride (2.9 mM).

In order to see the effect of NGF on the metabolism of chondromucoprotein, the femora precultivated for 2 days with regular BGJb-HW2 were incubated further for 12 h in the new medium containing carrier-free radioactive inorganic sulfate (2 µCi/ml) with or without NGF. ^{35}S-labelled chondromucoprotein was extracted by the method of Endo[9]. The medium was dialyzed against tap-water for 2 days and the radioactivity of the nondialyzable materials was determined as ^{35}S-labelled chondromucoprotein released into the medium during the 12 h incubation, because paper chromatographic analysis of the dialyzed medium showed that the ^{35}S-labelled nondialyzable substances in the medium are the mixture of chondroitin 4 sulfate and 6 sulfate (data not shown).

In order to see the effect of NGF on the synthsesis of other macromolecules, the cartilages precultivated for 2 days were incubated with [methyl-^{3}H] thymidine (1 µCi/ml, 10^{-6} M carrier thymidine), [6-^{3}H] uridine (1 µCi/ml, 10^{-6} M carrier thymidine and uridine) and L-[4,5-^{3}H] leucine (1 µCi/ml, 3.8×10^{-4} M carrier leucine) containing medium for 12 h with or without NGF. After the incubation, the femur was homogenized in 1 ml of cold 5% TCA by the use of a glass homogenizer. The homogenates were collected on Watman GF/C filter and precipitated radioactivity was measured by liquid scintilation counter.

Results and discussion. Table 1 shows that total synthesis of chondromucoprotein, as estimated by the sum of the radioactivity in the cartilage and that of the nondialyzable portion of the medium, was not changed by any concentration of NGF. However, 73% of the radioactive sulfate incorporated into the chondromucoprotein was released into the medium by 10 µg/ml of NGF, while in control culture only 1.3% of the total radioactivity was found in the medium. The stimulation of the release of chondromucoprotein by NGF was dependent on the dose: 18% of the total radioactivity was released by 1 µg/ml of NGF and no stimulation of the release was found by 0.01 µg/ml of NGF. These results suggest that NGF does not alter the chondromucoprotein synthesis but dramatically stimulates the degradation of this molecule.

10 µg/ml of NGF has little effect on RNA, DNA and protein synthesis in the cartilage, as estimated by the incorporation of radioactive uridine, thymidine and leucine respectively (table 2).

All these data show that NGF specifically stimulates the degradation of chondromucoprotein in the chick embryonic bone with little effect on the synthesis of the macromolecules such as chondromucoprotein, DNA, RNA and protein. Eisenbarth et al.[10] reported that NGF specifically inhibits the in vitro synthesis of chondromucoprotein in the chick embryonic pelvic rudiment as estimated by the incorporation of radioactive inorganic sulfate. The reason for the difference from my data is not clear but probably comes from the use of different material. They might have missed the degrading process because they had not determined the

Table 1. Effect of NGF on the synthesis and degradation of chondromucoprotein in 9 day chick embryo femur cultivated in vitro

	Radioactivity (cpm)			Degrading activity (%)**
	Cartilage (A)	Medium (B)	Total synthesis (A + B)*	
Control	886 ± 42***	16 ± 4	930 ± 46	1.6 ± 0.3
NGF (10 µg/ml)	228 ± 18	652 ± 81	880 ± 67	73.0 ± 3.8
Control	742 ± 26	37 ± 17	779 ± 38	4.4 ± 1.8
NGF (1 µg/ml)	574 ± 48	122 ± 21	694 ± 71	18.2 ± 3.4
Control	788 ± 36	22 ± 4	854 ± 44	2.5 ± 0.4
NGF (0.01 µg/ml)	802 ± 48	27 ± 10	882 ± 83	2.8 ± 0.8

The femora precultivated for 2 days were incubated further for 12 h with new medium containing carrier free radioactive inorganic sulfate (2 µCi/ml) with or without NGF to determine the incorporation into chondromucoprotein.

* Total synthesis of chrondromucoprotein was estimated by the sum of the radioactivity incorporated into cartilage and that of the nondialyzable portion of the medium; ** Degrading activity of chondromucoprotein was expressed as the ratio of the radioactivity in the medium to the total radioactivity in the cartilage and medium. (B/A + B × 100); *** Mean ± SE (n = 6).

radioactivity in the medium. The concentration of NGF necessary to affect the degradation of chondromucoprotein is much higher than those necessary to stimulate the growth of sympathetic and sensory ganglia. Rather high concentration of NGF was found in granulation tissue[6] and therefore NGF may play some role on the involution of granulation tissue by degrading the extracellular matrix. But the true physiological significance of this unique and specific effect of NGF in stimulating the degradation of cartilage chondromucoprotein is unknown at present.

Table 2. Effect of NGF on the uptake of ^{3}H-thymidine, ^{3}H-uridine and ^{3}H-leucine into TCA insoluble fraction in 9 day chick embryo femur cultivated in vitro

	Incorporated radioactivity (cpm)		
	^{3}H-thymidine	^{3}H-uridine	^{3}H-leucine
Control	26507±2647*	24517±995	15614±722
NGF (10 µg/ml)	23425±861	24441±988	12702±1205
% of control	88±6	101±4	83±7

The femora precultivated for 2 days were exposed to [methyl-^{3}H] thymidine, [6-^{3}H] uridine and L-[4,5-^{3}H] leucine containing medium for 12 h with or without NGF to determine the DNA, RNA and protein synthesis respectively. After the incubation, the femur was homogenized in 1 ml of cold 5% of trichloroacetic acid by the use of a glass homogenizer and the radioactivity of the trichloroacetic acid insoluble fraction was determined. * Mean ± SE (n = 6).

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Lead metabolism in lactation[1]

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Summary. A 2-fold increase in lead absorption was observed in lactating animals which received 2 mg Pb/l in drinking water. About one-half of the absorbed lead was transferred to the litters.

Lead is an environmental poison which can be transferred from mother to offspring through milk[3-5]. Various degrees of brain damage were produced in suckling rats with doses tolerated by their mothers, indicating that infants are a high risk group in relation to lead exposure[6-8]. A previous report from this laboratory showed an increase in the absorption of trace amounts of lead in lactating animals[9]. Considering that the gastrointestinal tract is the main route of entry of environmental lead into the body[3,10], the aim of this experiment was to investigate the effect of lactation on the absorption of lead from drinking water. A dose 20 times higher than the tentative limit of 0.1 mg Pb/l[11] was chosen because such an amount can be found in human diet[12].

Methods and results. The experimental group consisted of 10 lactating female rats each with a litter of 6. The control group consisted of an equal number of virgin rats of the same age. The animals were fed a stock laboratory diet (1.2% Ca and 0.8% P) and drank distilled water ad libitum. On the 12th day of lactation, 2 mg Pb/l were added to the drinking water marked with radioactive lead-203 (0.17 µCi/ml). After 2 days the lead-containing drinking water was discontinued and the animals were given distilled water for one more day. Individual urine samples were collected until the rats were killed on the 3rd day. The gastrointestinal tract of adult animals was removed to avoid the interference of nonabsorbed lead-203. Gamma-ray activity of the rat's carcass and urine, and, in the case of lactating rats, the whole body activity of their litters were determined in a single channel, twin crystal scintillation counter (Tobor, Nuclear Chicago, USA).

The apparent absorption expressed as the percentage retention of lead-203 in the carcass and urine, and, in the case of lactating rats, the whole body retention of their litters were the same in control and lactating rats (table). Lead-203 retention of the litters was almost 30% higher than the percentage dose retained in the carcasses of their mothers. When the results were corrected for a 2-fold increase in water consumption of lactating rats (58.4±2.0 vs 24.8±1.4 ml/day/rat) the absorption of stable lead was twice as high in lactating (3.6 µg Pb) as in control animals (1.5 µg Pb). Litter lead retentions (1.7 µg Pb) were as high as those for control animals.

Discussion. Increased lead absorption in lactating animals is most likely to be attributed to the well-documented morphological augmentation of the gastrointestinal tract during the lactation period[13,14]. However, the complexity due to

Administered dose of lead and lead-203, retention in carcass and litter and urinary excretion*

	Dose ^{203}Pb (%)	µg Pb
Controls		
Oral intake from drinking water	100	100±6
Carcass	1.0±0.1	1.0±0.3
Urine	0.5±0.1	0.4±0.4
Apparent absorption	1.5±0.6	1.5±0.6
Lactating		
Oral intake from drinking water	100	240±10
Carcass	0.5±0.03	1.2±0.3
Litter	0.7±0.2	1.7±1.1
Urine	0.3±0.1	0.6±0.6
Apparent absorption	1.6±0.7	3.6±1.4

* Each figure represents the mean of 10 animals ± SD.