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PRELIMINARY COMMUNICATION

PARADOXICAL DECREASE IN NOREPINEPHRINE CONTENT OF ADULT MOUSE SPLEEN AND HEART AFTER NEONATAL NERVE GROWTH FACTOR TREATMENT*

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The striking, but selective, effect of nerve growth factor (NGF) on the sympathetic-noradrenergic branch of the autonomic nervous system in mammals, after neonatal or adult treatment, is well documented [1-3]. In studies of short-term effects, where the interval between treatment and measurement is of the order of days at most, NGF administration elevates not only the norepinephrine (NE) specific activity (μg/g) and tissue NE content (μg/tissue) of noradrenergically innervated end-organs and/or ganglia, but also the activity of enzymes specifically involved in its (NE) synthesis. Daily treatment of newborn rats with 10 μg/g of NGF for 10 days enhances the growth and differentiation of neuroblasts in the superior cervical ganglion. Accompanying this characteristic morphological effect, was a selective induction (5 to 15-fold increase in specific and total activity, respectively) of tyrosine hydroxylase (EC 1.14.3, TH) and dopamine-β-hydroxylase (EC 1.14.2.1, DβH), two enzymes involved in NE synthesis in noradrenergic neuronal systems. In contrast, the activities of dopa-decarboxylase (EC 4.1.1.26) and monamine oxidase (EC 1.4.3.4, MAO), two more generally distributed enzymes present as well in other cell types, increase only in proportion to the volume growth of the superior cervical ganglion neurons

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[1,4]. As a result of this NGF-induced functional and structural hypertrophy, NE content in ganglia and several peripheral tissues measured soon after NGF treatment is increased well over control values, in some cases by as much as 300-400 per cent [1,3,5,6].

Nevertheless, there is little, if any, information on the significance of prior neonatal NGF treatment in the adult animal, since no study to date has followed up the long-term effects of early administration of NGF. Accordingly, some long-term effects were studied in adult mice after early NGF treatment. Nerve growth factor prepared from the submaxillary glands of male mice was administered subcutaneously (10 μg/g) to newborn male and female mice (Swiss-Webster) within 8 hr of birth, and continued daily for 10 consecutive days.* Litter-mate controls were similarly injected with an equal volume of phosphate-buffered (pH 7.2) physiological saline (PBS) employed as a vehicle for NGF.

Mice were sacrificed, at approximately 9 months of age, by cervical dislocation. Spleens, hearts and brains were rapidly but carefully dissected out, frozen and stored in liquid nitrogen for subsequent analysis. NE content was analyzed as described previously for endogenous NE [8]. Briefly, Alumina adsorption (columns) was used to purify tissue NE for fluorimetric assay by a modification of the trihydroxyindole method of von Euler and Lishajko [9]. All values are corrected for recovery of 80 per cent, determined by carrying standard solutions of NE through both Alumina adsorption and ferricyanide oxidation procedures. Tissue blanks were run for all samples in the oxidation.

Quite unexpectedly, both cardiac and splenic NE content and specific activity in both male and female mice after early NGF treatment were well below those measured in litter-mate controls [Table 1]. In fact, the results reported here appear to be qualitatively similar, biochemically, to results obtained after treatment of neonates with NGF-antiserum, where decreases in NE content of peripheral tissues are expected, and have been reported by many investigators [2]. The absence of this time-dependent reversal of NGF-induced short-term increases in tissue NE in brain can be attributed to the inability of the large NGF protein molecule to cross the blood-brain barrier.

To our knowledge, a change in adult mouse peripheral tissue NE content of the magnitude and direction found here after neonatal NGF treatment has not

*NGF was purified according to a modification of the procedure of Cohen[7]. Neonatal treatment included a few animals injected instead with a commercial NGF preparation (Wellcome Reagents MR60, 3 μg/g/day) for 6 consecutive days. Since the same results were obtained with these animals, they are included in this report.

Table 1. Norepinephrine Content of Adult Male and Female Mouse Spleen, Heart and Brain after Neonatal NGF Treatment

TISSUE		SPLEEN							HEART						BRAIN					
PARAMETER		% DEC.[a]	NE, ng/SPLEEN		WET WEIGHT[b]		NE, ng/gram		% DEC.	NE, ng/HEART		WET WEIGHT		NE, ng/gram		NE, ng/BRAIN		WET WEIGHT		NE, ng/gram	
TREATMENT			NGF	PBS	NGF	PBS	NGF	PBS		NGF	PBS	NGF	PBS	NGF	PBS	NGF	PBS	NGF	PBS	NGF	PBS
MALES	MEAN (n)	42.9 (5)	22.4 (5)	41.8 (5)	107 (5)	105 (5)	209 (5)	396 (5)	30.3 (6)	83.7 (6)	122 (6)	214 (6)	218 (6)	387 (6)	552 (6)	176 (4)	177 (4)	502 (4)	506 (4)	349 (4)	350 (4)
	± S.E.M.	8.9	3.0	7.0	9.1	9.2	19	49	7.3	13	17	19	16	43	51	10	7.3	16	19	18	4
	S_1[c]	-	.0125<p<.025		N.S.		.0025<p<.005		-	.05<p<0.1		N.S.		.0125<p<.025		N.S.		N.S.		N.S.	
FEMALES	MEAN (n)	61.9 (5)	17.2 (5)	44.9 (5)	109 (5)	98.6 (5)	161 (5)	459 (5)	22.3 (8)	87.7 (8)	113 (8)	155 (7)	167 (7)	579 (7)	701 (7)	177 (5)	169 (5)	507 (5)	492 (5)	371 (5)	342 (5)
	± S.E.M.	0.7	1.4	3.4	5.1	7.6	18	30	3.6	6.9	7.0	5.5	6.8	35	32	18	16	5.2	13	26	26
	S_1	-	p<0.0005		N.S.		p<0.0005		-	.001<p<.0125		N.S.		.0125<p<.025		N.S.		N.S.		N.S.	
MALES & FEMALES	MEAN (n)	-	19.8 (10)	43.4 (10)	-	-	185 (10)	427 (10)	-	86.0 (14)	117 (14)	-	-	491 (13)	632 (13)	-	-	-	-		
	± S.E.M.	-	1.8	3.7	-	-	15	29	-	6.6	8.0	-	-	38	35	-	-	-	-	-	-
	S_1	-	p<0.0005		-		p<0.0005		-	.0025<p<.005		-		.005<p<.01		-		-		-	
	S_2[d]	p<.05	N.S.		N.S.		N.S.		N.S.	N.S.		p<.01	p<.01	p<.005	p<.025	N.S.		N.S.		N.S.	

Values are expressed on both a total content (ng/tissue) and specific activity (ng/g) basis. (a) % DEC: $\frac{\text{NE/tissue after PBS} - \text{NE/tissue after NGF}}{\text{NE/tissue after PBS}}$ (b) wet weight: expressed in mg; one value for female heart is omitted because of a blotting error. (c) S_1: significance of difference (t-test), experimental (NGF) treatment vs control (PBS) treatment. (d) S_2: significance of difference (t-test), males vs females.

previously been reported. Nevertheless, two recent publications, concerning a time-dependent reversibility of the NGF effect, may be related to our present finding. Edwards et al. [10] have reported that, after an initial NGF-induced increase in mouse superior cervical ganglion weight (cell number and cell size) observed within 1 day after a 6-day neonatal injection schedule (5 μg/g/day), values appear to return by 44 days to those seen in 44-day-old control animals.

Similarly, Hendry [11] has shown that the increased production of tyrosine hydroxylase observed immediately after neonatal NGF administration (3 μg/g/day for 6-10 days) returns to control values 1-2 months later. In addition, preliminary results indicate that, by 5 months of age, NE content and ^{3}H-NE uptake of peripheral tissues did not differ between early NGF (1 μg/g)- and saline-injected mice [R. J. Campbell, E. A. Stone and L. V. DiCara, unpublished observation].

Although no definitive explanation of these results is yet possible, two plausible directions might be mentioned. Abnormally high levels of exogenous NGF, widely distributed rather than more specifically located endogenously in various end-organs during the neonatal period, would result from daily NGF injections. It is possible that this elevated NGF shuts off, via a feedback mechanism, or antagonizes, via production of inactivating antibodies, (development of) the physiological NGF-producing mechanisms subserving development and

maintenance of peripheral noradrenergic neuronal systems. A second possibility depends upon the NGF-altered balance between the NE-synthesizing enzymes (TH and DβH) and the intracellular NE-deaminating enzyme (MAO): noradrenergic nerve terminal destruction may occur when NE metabolism is altered such that the ratio $\frac{TH \times D\beta H}{MAO}$ is increased, with the resultant excess dopamine and/or dopamine-β-hydroxylase leading to dopamine-ortho-quinone formation in the terminals. In this regard, it is interesting to note that growth and extension of neurites in sympathetic neuroblasts in vitro are adversely affected by higher than optimal concentrations of NGF [12].

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