SPECIFIC BINDING SITES FOR ^{125}I-NERVE GROWTH FACTOR IN PERIPHERAL TISSUES AND BRAIN

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SUMMARY--Specific binding of ^{125}I-nerve growth factor (NGF), defined as that part of the total binding of the iodinated derivative displaced by 15-30 µg/ml native NGF, is found at significant levels in many peripheral tissues of chick embryos and rats. Destruction of the sympathetic innervation of tissues by treatment of newborn rats with guanethidine does not materially alter the ^{125}I-NGF specific binding capacity of tissues, indicating that these binding sites for NGF are part of the tissues themselves and not a property of the sympathetic nerve terminals which innervate them. Specific binding of ^{125}I-NGF which is also resistant to guanethidine treatment exists in chick embryonic and rat brain. The time course of the development of this specific binding in chick embryonic heart and brain suggests a developmental role for these peripheral and central nervous system NGF binding sites.

INTRODUCTION--Nerve growth factor (NGF) is a hormone-like protein which functions during the embryonic development and subsequent maintenance of the sympathetic nervous system of vertebrates (1,2). Its recognized target tissues, in which it stimulates nerve fiber outgrowth and maintains a morphologically and metabolically differentiated state, include sympathetic neurons and a population of neurons in dorsal root (sensory) ganglia (1,3). Structural and functional similarities between NGF and insulin (proinsulin) (4,5) suggested that the mechanism of action of these two proteins might be similar. This hypothesis was substantiated by the demonstration of surface membrane receptors for NGF on sympathetic and sensory neurons by means of insolubilized NGF (NGF-Sepharose) (6) and the properties of the interaction of NGF with this receptor have been

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studied with ^{125}I-NGF prepared by a solid phase iodination technique[2]. The interaction of ^{125}I-NGF with its receptor in these neurons is a complex process which displays multiple affinities, possibly due to a negatively cooperative interaction of NGF receptors[2]. The properties of the NGF-receptor interaction are very similar to those of the insulin-receptor interaction (7-10).

This communication reports the identification of specific binding sites for ^{125}I-NGF in peripheral organs and brain of chick embryo and rat, which are not on sympathetic nerve endings within the tissues. The tissue distribution and development pattern of this specific peripheral ^{125}I-NGF binding suggests a general correlation with the degree of sympathetic innervation which the tissue receives and suggests that these binding sites may have a functional role in the development of peripheral innervation. The specific binding of ^{125}I-NGF in brain correlates with recent reports of functional effects of NGF on the central nervous system (11,12).

METHODS--Tissues of chick embryos or rats were obtained by rapid dissection of freshly sacrificed specimens and disrupted by homogenization in a large clearance Ten-Broeck homogenizer. Particulate material (whole cells and membrane fragments) was collected by centrifugation at 2,000 x g for 20 min and resuspended in Hank's salt solution buffered with Hepes (Sigma) at pH 7.40 containing 10 mg/ml BSA. ^{125}I-NGF (2.5S) was prepared, and its specific binding to tissue suspensions was assayed, as described[2]. Specific binding is defined as that amount of the total binding displaced by 30 µg/ml native NGF. Rats, treated since birth with guanethidine, were the generous gift of Dr. Phillip Needleman.

RESULTS--Table 1 shows the relative levels of specific ^{125}I-NGF binding to sympathetic ganglia, brain and some peripheral tissues of 13 day chick embryos and newborn rat. Although the level of specific binding is

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TABLE 1

Binding of ^{125}I-NGF to tissues of newborn rat and 13 day chick embryo[a]

	Specific Binding (cpm/μg protein)	
Tissue	Chick[b]	Rat[c]
Sympathetic ganglia	2050	1790
Heart	687	215
Brain	442	93
Liver	309	45
Adrenal[d]	n.d.	399
Red blood cells[d]	n.d.	2

[a]Specific activity of this preparation was 1374 cpm/fmole.

[b]9.8×10^{-10} M ^{125}I-NGF.

[c]3.9×10^{-10} M ^{125}I-NGF.

[d]n.d., not determined.

highest in sympathetic ganglia of both organisms, significant levels of specific binding exist in all other tissues shown except red blood cells.

Table 2 shows the results of more extensive experiments with 6 week and 4 month old rats. In the columns headed control, it is seen that all tissues of normal animals examined, with the exception of red blood cells, had significant levels of specific ^{125}I-NGF binding. In both the 6 week and 4 month old rat, the highest level of binding outside the sympathetic ganglia is found in blood vessel (abdominal aorta) and thus much or all of the binding in tissues such as skeletal muscle may be due to their vascularization.

TABLE 2

Specific binding of ^{125}I-NGF to tissues of control and guanethidine-treated rats

	Specific Binding (cpm/μg)			
	6 week[a]		4 month[b]	
Tissue	Control	Guanethidine-treated	Control	Guanethidine-treated
Sympathetic ganglia	737	174	656	562
Heart	10.2	9.8	58.7	57.4
Brain	7.2	10.5	114	156
Liver	4.0	6.0	19.9	10.1
Spleen	17.0	18.0	47.8	27.4
Adrenal	50.7	61.4	65.9	109
Abdominal aorta	130	144	414	411
Uterus	100	140	172	100
Kidney[c]	n.d.	n.d.	32.8	17.1
Diaphragm[c]	n.d.	n.d.	51.7	55.9
Skeletal muscle[c]	n.d.	n.d.	31.6	53.8
Red blood cells	0	1.2		

[a]The specific activity of this preparation was 700 cpm/fmole and the concentration of ^{125}I-NGF was 5.9×10^{-10} M.

[b]The specific activity of this preparation was 512 cpm/fmole and the concentration of ^{125}I-NGF was 2.1×10^{-9} M.

[c]n.d., not determined.

To determine whether the specific ^{125}I-NGF binding observed in these tissues was due to the presence of sympathetic nerve endings, the tissues of animals whose sympathetic nerve terminals had been destroyed with guanethidine were assayed for specific ^{125}I-NGF binding. These animals

had received daily injections of guanethidine for the first 20 days after birth. They were devoid of sympathetic function by physiological criteria and the levels of catecholamines in their peripheral organs had been diminished by greater than 90%[3]. As seen in Table 2, the level of specific ^{125}I-NGF binding in the sympathetic ganglia of the 6 week rat was greatly diminished while in the 4 month rat not as great a difference was seen. This may indicate that some regeneration of the sympathetic ganglia occurs with time. It should be noted, however, that at both ages, the size of the sympathetic chains was greatly reduced. In the case of the 6 week rats, no tissue from the guanethidine-treated animal showed a significantly decreased level of specific ^{125}I-NGF binding. The apparent increase in specific binding with guanethidine treatment is probably due to the lower concentrations of tissue protein used in the assay of specific binding[4]. For the 4 month rats, the majority of tissues display the same or elevated specific ^{125}I-NGF binding with guanethidine treatment, while liver, spleen, uterus and kidney show a decrease of about 50% in the level of specific binding. In no case has the guanethidine treatment affected the level of specific ^{125}I-NGF binding to the extent that it destroys sympathetic nerve terminals. These results indicate that, in all tissues, a significant fraction of the total specific ^{125}I-NGF binding is to structures other than sympathetic nerve terminals. In addition, detailed studies of the properties of these specific NGF binding sites in chick embryo heart and brain[5] indicate that their affinities and kinetic properties with respect to ^{125}I-NGF are very similar to those of the NGF-receptor interaction of sympathetic and dorsal root ganglia[2].

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[3] Johnson, E.M. and Needleman, P., personal communication

[4] Pulliam, M.W. and Szutowicz, A., unpublished experiments

[5] Frazier, W.A., Boyd, L.F., Szutowicz, A., Pulliam, M.W. and Bradshaw, R.A. manuscript in preparation

The appearance and variation during embryonic development of specific ^{125}I-NGF binding sites in chick embryo heart and brain has been studied. In these experiments, the amount of tissue suspensions of organs at different stages of development taken for assay was adjusted to obtain identical concentrations of tissue protein. The results of this study are presented in Fig. 1 as specific ^{125}I-NGF per µg of tissue protein and per one heart or brain. To directly compare the results of two experiments with heart and three experiments with brain, all values were compared to 100% at the highest number obtained and the results averaged.

As seen in Fig. 1, significant levels of specific ^{125}I-NGF binding appear very early in development. In both heart and brain, the binding per µg of tissue protein was maximal at 7 to 8 days of development and declined thereafter. The total specific binding in both organs, however, increases throughout development and was maximal at 16 days, the latest age studied (hatching occurs on day 21). The rate of appearance of specific ^{125}I-NGF binding is markedly different in heart and brain. In heart there is a slow increase from day 6 (1%) to day 10 (13%) and a rapid increase beyond day 10. In brain, however, the binding develops much more rapidly between day 6 to day 9 where it is already more than half maximal. The levels of binding in both organs tends to plateau from 9 to 10 days, but at different levels.

DISCUSSION--The results reported here indicate that specific, high affinity binding sites for NGF exist in many peripheral organs and the central nervous system of chick embryo and rats. These binding sites do not exist primarily on sympathetic nerve terminals within the tissues, although there is a general correlation of the level of NGF binding with degree of sympathetic innervation. The different developmental pattern of total specific NGF binding in heart and brain indicates, even at this rather gross level of comparison, a temporal specificity. It is of interest that the highest relative concentration of NGF binding sites

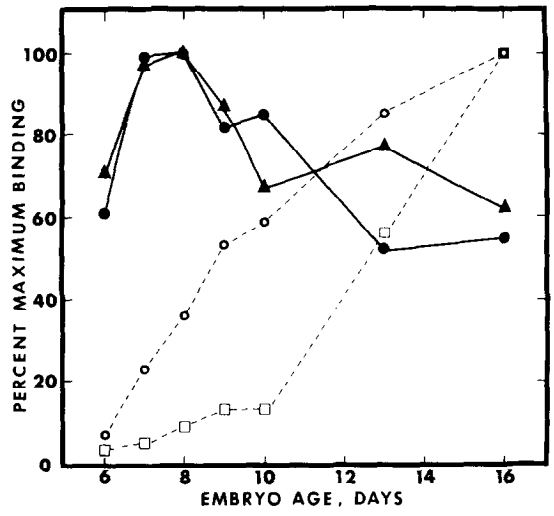

Figure 1. The change in specific ^{125}I-NGF binding capacity of chick embryo heart and brain as a function of developmental stage (age in days) of the embryo. The solid lines indicate the percent of maximum specific binding per μg tissue protein for heart (▲) and brain (●). The maximum occurs at 8 days of development for both heart and brain, and the actual levels of binding were 0.2 fmole/μg and 0.45 fmole/μg respectively at comparable ^{125}I-NGF concentrations. The dashed lines indicate the percent of maximum specific binding per one heart (□) or brain (○) at a given stage of development. The actual maximum values of specific binding per organ were about 1 pmole for both heart and brain at comparable concentrations of ^{125}I-NGF. Two experiments with heart were performed at 3.7 and 4.7 x 10^{-9} M ^{125}I-NGF and three experiments with brain at 1.3, 2.5 and 3.9 x 10^{-9} M ^{125}I-NGF.

occurs at 8 days of development in both heart and brain, a situation identical with the temporal distribution of NGF receptors in dorsal root ganglia[2] which exhibit the maximal morphological response to NGF at this time (1,2). This analogy in the time of appearance of the greatest concentration of NGF binding sites in brain and peripheral organs and in dorsal root ganglia, may indicate an analogous temporally dependent function of NGF in these tissues.

The demonstration that specific binding sites for NGF exist in brain is particularly significant in view of reports of functional effects of NGF on the catecholaminergic neurons of brain (11,12). It is likely that these effects are mediated by the interaction of NGF with the binding sites described in this report and that these sites represent

NGF receptors. At the present time, the function and physiological significance of the NGF binding sites of peripheral organs is unknown. However, their distribution and properties suggest a functional role in the development of sympathetic innervation, both in directing the growth of sympathetic nerve fibers to end organs and in maintaining functional innervation.

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