Vol. 88, No. 2, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 28, 1979 Pages 563-568

NERVE GROWTH FACTOR INFLUENCES SODIUM ION EXTRUSION FROM CHICK EMBRYONIC DORSAL ROOT GANGLIONIC NEURONS

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Received March 5,1979

SUMMARY. Cell dissociates from embryonic chick spinal ganglia, incubated for several hours with sodium-22, accumulated six times more radioactivity in the absence than in the presence of nerve growth factor. Cells incubated without nerve growth factor and sodium-22 for six hours, and then presented with sodium-22 also accumulated radioactivity more readily. Delayed presentation of nerve growth factor to sodium-22 preloaded cells caused a rapid extrusion of radioactivity. Intact spinal and sympathetic ganglia from chick embryo showed similar qualitative behaviors. Thus, a critical action of nerve growth factor may be to enhance a sodium-extrusion mechanism in its target neurons.

INTRODUCTION

Nerve growth factor (NGF) is essential for the normal growth, maintenance, and development of sympathetic and sensory neurons (1,2). The mechanism whereby NGF accomplishes this trophic role remains to be elucidated. Recent evidence has indicated that NGF may act to regulate the permeation properties of chick embryonic dorsal root ganglionic cells. The uptake activity of these ganglionic cells *in vitro* toward hexoses, certain amino acids and nucleosides is maintained only in the continuous presence of NGF and is fully recoverable if NGF is added to the cells within the first several hours (3-5). We have recently demonstrated that (i) the NGF-dependent portion of the total specific hexose uptake is also dependent on the presence of Na^+ ions, and is inhibited by ouabain and dinitrophenol, and (ii) uptake activity for other substrates which exhibit NGF regulation is likewise Na^+ sensitive (6,7).

These findings encouraged speculation on a possible link between sodium gradients across the neuronal membrane and the mode of action of NGF. With this in mind, we have attempted to ascertain whether NGF could indeed influence the movement of Na^+ ions across the membrane of its target cells.

Abbreviations: NGF, Nerve Growth Factor (7S complex); B.U., Biological Unit for nerve growth factor; DRG, dorsal root ganglia; ChCl, choline chloride.

0006-291X/79/100563-06$01.00/0

MATERIALS AND METHODS

Sodium-22 (carrier-free $^{22}NaCl$) was purchased from New England Nuclear. Nerve growth factor, 7S complex and beta subunit species were prepared according to the technique of Varon et al. (8). Glass fiber filter discs (GF/C) were purchased from Whatman.

Accumulation of $^{22}Na^{+}$ by dorsal root ganglionic cells. Dorsal root ganglia (DRG) from 8-day old White Leghorn chick embryos were dissected out, collected in Ca^{2+}- and Mg^{2+}-free Hank's balanced salt solution, and dissociated in a Tris-Hepes albumin medium (medium-A) containing 40mM Tris-Hepes pH7.4, 140mM NaCl, 5mM KCl, 1mM $MgCl_2$, 0.1mM $CaCl_2$, and 1% (W/V) bovine serum albumin, all as described previously (6,7). The cell suspension, following dilution to 1.0×10^6 cells/ml with medium-A, was divided into two equal volumes. One portion received 7S NGF at a concentration of 1 Biological Unit (B.U.)/ml, and the other an equal volume of medium-A. Concentrations of NGF for both the 7S and beta species were expressed in B.U./ml, that is, in terms of concentrations required to elicit a biological response in the traditional bioassay (8). Sodium-22 (as $^{22}NaCl$) was then added to the cell suspensions at 7.5μCi/ml, and incubation carried out at 37°C with shaking. At various times over an 8 hr period, aliquots (0.1ml) of cell suspension were transferred to moist GF/C glass fiber filter discs (2.4cm diameter) under suction. The filters were washed with 5ml of ice-cold medium-A, dried on a warm hot plate and counted via liquid scintillation techniques (5).

Cell suspensions were also incubated for 6 hr at 37°C, with or without NGF (1 B.U./ml), in medium-A where NaCl had been replaced by an equal concentration of choline chloride (ChCl). Following this incubation, the cells were pelleted by centrifugation at 400xg for 3 min and resuspended in an equal volume of medium-A (with NaCl, with or without NGF) containing 7.5μCi/ml of carrier-free $^{22}NaCl$ (medium prewarmed to 37°C). At different times after returning the cells to the 37°C bath, aliquots (0.1ml) were transferred to moistened GF/C filters and processed as described above. Blanks consisted of 0.1ml of medium-A having $^{22}NaCl$ but no cells.

Efflux of $^{22}Na^{+}$ from preloaded dorsal root ganglionic cells. A DRG cell dissociate was prepared as described in the preceeding section. The dissociate was incubated with $^{22}NaCl$ (7.5μCi/ml) and no NGF for 6 hr at 37°C. The cell suspension was centrifuged at 400 x g for 3 min, and the cell pellet resuspended in an equal volume of medium-A having no $^{22}NaCl$. The resuspended cells were divided into three equal portions, and received 7S NGF at 1 B.U./ml, beta NGF at 5 B.U./ml, or an equal volume of medium-A alone. At different times after incubation at 37°C, aliquots (0.1ml) of cell suspension were transferred to moistened GF/C filters, washed and counted for radioactivity as described above.

RESULTS AND DISCUSSION

Incubation of dorsal root ganglionic cells with $^{22}Na^{+}$ in the absence of NGF over an 8 hr period resulted in the accumulation of radioactivity to a far greater extent (about 6-fold after 6 hr), when compared to cells continuously maintained with NGF at 1 B.U./ml (Fig. 1A). The $^{22}Na^{+}$ accumulation which accompanied the lack of NGF appeared to reach a plateau level after 6-8 hr.

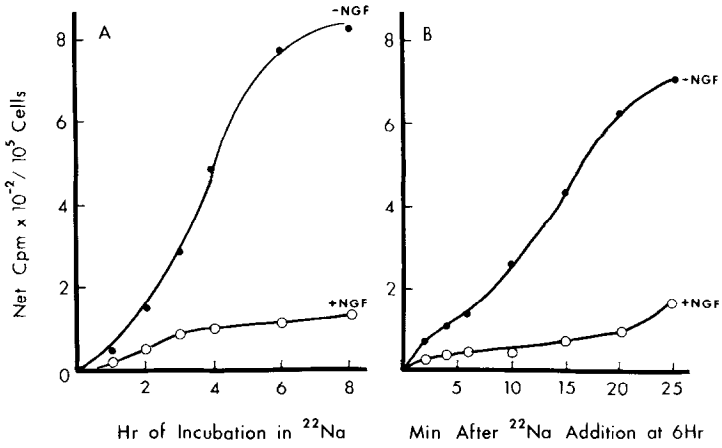

Fig. 1. Accumulation of $^{22}Na^+$ by DRG cells during (A) or following (B) incubation with or without NGF. (A): Dissociated DRG cells at 1.0 x 10^6 cells/ml were incubated with ^{22}NaCl (7.5μCi/ml) at 37°C with 1 B.U./ml of NGF or no NGF. At the indicated times 0.1 ml of cell suspension was transferred to GF/C filter discs, washed and counted as described in Materials and Methods. The zero time value was subtracted before plotting. (B): The above cell suspension was incubated in medium-A containing ChCl in place of NaCl, with 1 B.U./ml of NGF or no NGF. After 6 hr at 37°C cells were transferred to standard medium-A (with or without NGF) containing 7.5μCi/ml of ^{22}NaCl. At different times aliquots (0.1 ml) were transferred to GF/C filters and processed as in (A). Blanks contained 0.1 ml of medium-A with ^{22}NaCl but no cells. Each point represents the average of duplicate measurements in each of two separate experiments.

In some experiments, cells were incubated in medium-A where the NaCl had been replaced by an equal concentration of ChCl. After 6 hr with or without NGF (1 B.U./ml) the cells were transferred to standard medium-A with $^{22}Na^+$. As seen in Fig. 1B, cells deprived of NGF for 6 hr accumulated $^{22}Na^+$ much more extensively than cells continuously maintained with NGF. A 4- to 6-fold difference was detectable within minutes of the $^{22}Na^+$ presentation. Thus, the consequences of NGF deprivation on the machinery controlling sodium accumulation develop over several hours (Fig. 1A), even in the actual absence of sodium ions (Fig. 1B), but can then be expressed within minutes of exposure to extracellular sodium.

The above findings are subject to two alternative interpretations, namely: i) NGF promotes a Na^+ extrusion mechanism, needed to compensate for

normally occurring Na^+ influxes, or ii) NGF regulates the entry of Na^+ into its target cells (i.e., maintains a low permeability of their plasma membrane to Na^+). Dorsal root ganglionic cells were allowed to accumulate $^{22}Na^+$ in the absence of NGF (as in Fig. 1A) and then transferred to $^{22}Na^+$-free medium-A. These cells should release their radioactive load i) more rapidly in the presence of NGF if NGF activates a sodium extrusion system, or ii) less (or equally) rapidly in the presence than in the absence of NGF, if NGF reduces membrane permeability to sodium ions. The results of such an experiment are presented in Fig. 2. In the absence of NGF, release of radioactivity is slow and linear with time. The presence of 7S NGF (1 B.U./ml) leads to a loss of about 90% of the initial radioactivity content in about 10 min (compared to 30% in NGF-free medium). Use of pure beta NGF (5 B.U./ml) produces very similar results, demonstrating that they are truly NGF effects. The need for greater amounts of beta than 7S NGF species to achieve equivalent results has already been observed with regard to hexose uptake (3-5). It is important to note that, with 1 B.U./ml of 7S NGF, the effect on Na^+ efflux is displayed with less than a 2-min lag, a much shorter delay than the 5-min lag observed under identical circumstances for the reactivation of hexose (or uridine) uptake activity (3-5).

Several experiments were also carried out using intact chick embryo sensory and sympathetic ganglia. As with dissociated DRG, intact sensory ganglia accumulated greater amounts of $^{22}Na^+$ in the absence, than in the presence of NGF. Extrusion of $^{22}Na^+$ from preloaded, NGF-deprived sensory ganglia was also enhanced upon addition of NGF. Sympathetic ganglia from 11-day chick embryos behaved in a qualitatively similar fashion to the intact sensory ganglia.

We had previously shown that, in DRG cells, NGF controls uptake mechanisms for certain exogenous substrates, which are also dependent on a Na^+ gradient for their operation (6,7). The two requirements, for Na^+ gradient and NGF, by such mechanisms could have been independent from each other, or

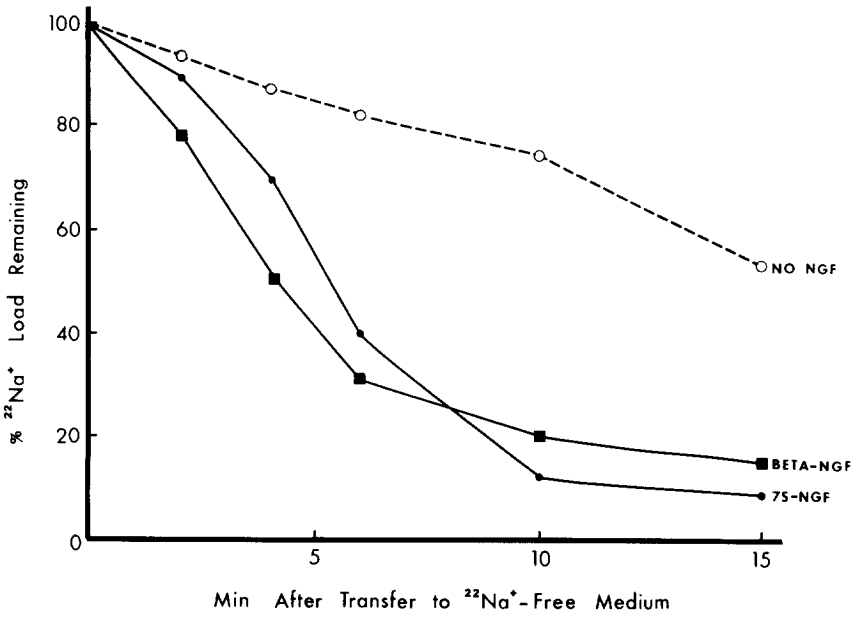

Fig. 2. Efflux of $^{22}Na^+$ from preloaded DRG cells following NGF addition. A DRG cell dissociate at 1.0 x 10^6 cells/ml was incubated with $^{22}NaCl$ (7.5μCi/ml) for 6 hr at 37°C in the absence of NGF. The cells were then transferred to medium-A without $^{22}NaCl$, but with 7S NGF at 1 B.U./ml, beta NGF at 5 B.U./ml, or no NGF. At different times aliquots (0.1 ml) were removed to GF/C filters, washed and counted as described in Materials and Methods. Each point represents the average of duplicate measurements in each of two (beta NGF) or three (7S NGF) separate experiments. Values are expressed as a percentage of the radioactivity in the cells immediately following transfer to $^{22}Na^+$-free medium.

one subordinate to the other. It becomes clear from the present data that the latter is the case. At least in these DRG cells, NGF controls the accumulation of intracellular Na^+, which permits the maintenance of an appropriate Na^+ gradient across the target cell membrane, and this in turn provides the driving force for a Na^+-coupled intake of hexose, among other substrates. The consequences of this NGF action on the intake of critical substrates may be all that is required to explain the trophic functions of NGF on its target neurons (1-7). On the other hand, the demonstration that NGF controls the levels of intracellular Na^+ (as well as the Na^+ gradient) opens up several new possibilities for NGF to affect other Na^+-sensitive cellular performances.

The mechanism by which NGF regulates intracellular Na^+ levels in DRG cells is only partially clarified by the present study. Nerve growth factor decreases Na^+ accumulation inside its target cells, and apparently does so by an extrusion mechanism rather than by membrane permeability changes. The enhanced extrusion of Na^+ by hormones or other regulatory factors is not without precedent, as insulin has been reported to do so in frog skeletal muscle, possibility by unmasking latent Na^+, K^+-ATPase pump sites (9).

The nature of the sodium extrusion mechanism which is influenced by NGF remains unknown. The classical Na^+-K^+ pump (Na^+, K^+-ATPase) is usually associated with maintaining the transmembrane sodium gradient for Na^+-dependent hexose and amino acid transport systems in several other cell and tissue types (10,11). On the other hand, Na^+ extrusion could still be a secondary consequence of an NGF action primarily directed to other ionic species, such as K^+, Ca^{2+}, or H^+. Further experimentation is clearly required to determine the primary molecular target of NGF action.

ACKNOWLEDGEMENT. This work was supported by USPHS grant NS-07606 from the National Institute of Neurological and Communicative Diseases and Stroke.

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