

Changes in plasma nerve growth factor levels in older adults associated with chronic stress[☆]

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Abstract

Evidence indicates that the actions of nerve growth factor (NGF) reach beyond the nervous system and might modulate immune function. Based on reports that blood NGF rises following the acute stress of parachute jumping, we investigated whether exposure to a chronic stressor, caregiving for a cognitively impaired spouse, could alter the levels of blood NGF. High perceived stress and depression in caregivers (vs. well-matched controls) were associated with elevated blood NGF. These data suggest that exposure to this chronic stressor can alter the concentrations of circulating NGF, and that psychological stress can induce changes in NGF concentrations in older adults. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Immune responses can be influenced through a complex network of bi-directional signals involving the nervous, endocrine, and immune systems. Psychological stress can dysregulate immune responses as a consequence of altered interplay among the systems. Neuroendocrine hormones released by the activation of the hypothalamic–pituitary–adrenal (HPA) axis, catecholamines, and other hormones and neuropeptides associated with the sympathetic nervous system play a role in these complex interactions. These pathways produce biologically important molecules that,

directly or indirectly, modulate immune function (Rabin, 1999). The presence of sympathetic fibers in lymphoid tissues suggests a possible venue for trafficking neural messages and initiating signaling cascades in immune cells (Felten et al., 1987).

The neurotrophin nerve growth factor (NGF) is a potent trophic factor for sympathetic neurons and, as such, is in a key position to control the responsiveness of immune-competent cells (Levi-Montalcini et al., 1995). Furthermore, NGF, via the hypothalamus (Scaccianoce et al., 1993), can activate the HPA axis (Otten et al., 1979) and play a role in adaptive responses. More importantly, there is evidence that NGF might be an autocrine/paracrine factor for the development and regulation of immune cells (Levi-Montalcini et al., 1995). NGF is produced by T and B lymphocytes (Torica et al., 1996; Ehrhard et al., 1993; Lambiase et al., 1997; Santambrogio et al., 1994) which display functional NGF receptors (Franklin et al., 1995; Brodie and Gelfand, 1992; Otten et al., 1989; Ehrhard et al., 1994; Kittur et al., 1992; Morgan et al., 1989). Furthermore, NGF promotes the proliferation and differentiation of T and B lymphocytes (Brodie and Gelfand, 1992; Otten

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Abbreviations: HPA, hypothalamic–pituitary–adrenal axis; NGF, nerve growth factor; ACTH, adreno-corticotrophic hormone

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et al., 1989; Thorpe and Perez-Polo, 1987), and acts as a survival factor for memory B lymphocytes (Torica et al., 1996).

Stressors affect immune responses and have been implicated in the expression and severity of immunological disorders. Neurohormones, catecholamines, and cytokines have been considered as mediators of these stress-induced changes. Over the past two decades, evidence has accumulated that circulating NGF is altered after exposure to stressful events (Alleva et al., 1996a,b; Aloe et al., 1994), as well as in various immunological diseases (Bonini et al., 1996). These observations have promoted the hypothesis that NGF might serve as one of the links between neuroendocrine and immune elements, translating environmental messages into immune responses. For example, aggressive behavior in mice is accompanied by a massive release of NGF into the blood (Alleva et al., 1993, 1996a,b; Lakshmanan, 1986, 1987), elevated levels of NGF mRNA and protein in the hypothalamus (Spillantini et al., 1989), enhanced secretion of ACTH and glucocorticosteroids (Ottien et al., 1979), and adrenal gland hypertrophy (Alleva et al., 1993).

It has been shown that the concentration of blood NGF increased during the stressful event of parachute jumping (Aloe et al., 1994). In this study, we investigated whether a chronic stressor, caregiving for a spouse with a progressive dementia, could produce changes in the levels of NGF in the blood. Dementia caregiving can provoke significant dysregulation of cellular immunity and neuroendocrine homeostasis, and these changes have health consequences (Bauer et al., 2000; Kiecolt-Glaser et al., 1991, 1995, 1996; Wu et al., 1999).

2. Materials and methods

2.1. Subjects

Subjects were part of a larger longitudinal study on stress and health in older adults (Kiecolt-Glaser et al., 1991; Esterling et al., 1994). Spousal caregivers had to be providing 5 h or more of care per week; control subjects were recruited through newspaper advertisements, church groups, notices posted in senior citizen centers, and refer-

Table 1

Level of depression and perceived stress in caregivers

	Controls (mean \pm SEM)	Caregivers (mean \pm SEM)
Depression	1.67 \pm 0.41	5.87 \pm 0.7*
Perceived stress	5.80 \pm 1.06	19.13 \pm 1.53*

Depression was assessed using the Beck Depression Inventory-Short Form and perceived stress was assessed using the Perceived Stress Scale.

* $p < 0.001$ compared with controls.

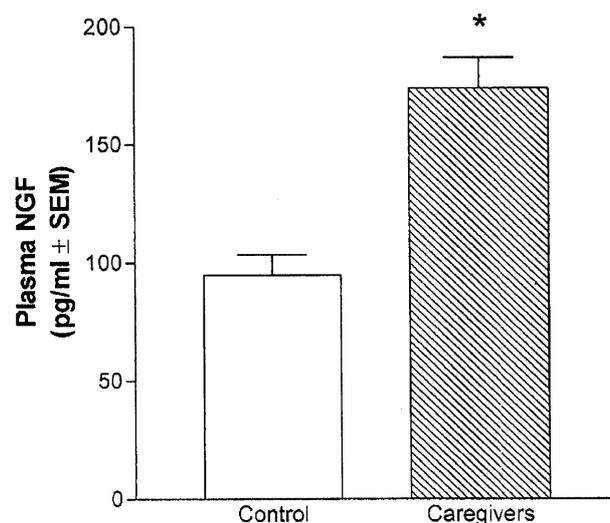


Fig. 1. Concentration of plasma NGF in controls and caregivers with high perceived stress and depression scores. Plasma NGF was measured as described in the Materials and Methods. $N = 15$ caregivers, 15 controls. * $p < 0.001$ compared with controls.

als from other participants. Subjects were excluded if they had immunologically related health problems. The study reported here used a subset of caregiver and control subjects from the longitudinal project. The study included 15 caregivers and 15 controls, all female. Subject selection was designed to maximize group differences in depression and perceived stress; caregivers reporting high distress and controls reporting low distress were selected. There were no significant differences between caregivers and controls on age (69 ± 2 vs. 70 ± 2 years), race (93% Caucasian in each group), education, or income. Among controls, 53% was currently married. The inclusion of divorced and widowed control subjects worked against confirmation of the experimental hypotheses because intact marriages are associated with lower rates of psychopathology and less morbidity and mortality (House et al., 1988).

2.2. Evaluation of depression, stress, and health behaviors

Subjects' distress was evaluated using the Beck Depression Inventory-Short Form (Beck and Beck, 1972) and the Perceived Stress Scale (Cohen et al., 1983), which provide information on the severity of subjects' affective and cognitive depressive symptoms and the degree to which individuals appraise situations in their life as stressful. Assessment of health-related behaviors included medication use, smoking, caffeine intake, alcohol intake, amount of sleep in the last 3 days, and weight changes in the last 2 weeks. Plasma albumin levels provided objective information on the nutritional status of subjects (Glaser et al., 1990).

2.3. Blood NGF levels

Blood for all subjects was drawn early in the morning, between 8:00 AM and 10:00 AM, to control for diurnal variation. Caregivers and controls were run in mixed groups so that blood was simultaneously collected and assayed from both groups. Plasma was collected from heparinized whole blood, supplemented with 20 $\mu\text{g}/\text{ml}$ aprotinin, and stored at -86°C until assayed. NGF levels were measured by using an ELISA (Boehringer Mannheim), according to the instructions of the manufacturer. All plasma samples were assayed in duplicate and run at the same time. The study was blinded; the caregiver vs. control subject status of the blood samples was not known by those performing the NGF assay.

2.4. Statistical analysis

Differences between control and caregiver subjects in psychological variables and plasma NGF levels were tested using Students' *t*-test. The association between psychological distress and plasma NGF was examined using Pearson's correlation. Differences between groups were considered statistically significant at $p < 0.05$.

3. Results

Caregiver and control subjects were selected so that differences in distress, as assessed by the Beck Depression Inventory and the Perceived Stress Scale, were maximized. Consistent with selection criteria and with previous reports (Kiecolt-Glaser et al., 1991, 1995, 1996), caregivers reported significantly higher depressive symptoms (care-

givers 5.87 ± 0.7 , controls 1.67 ± 0.41 , $p < 0.001$) and perceived stress (caregivers 19.13 ± 1.53 , controls 5.80 ± 1.06 , $p < 0.001$) than controls (Table 1). The concentration of plasma NGF in the caregivers (174.07 ± 13.06 pg/ml) was approximately two times the value found for controls (94.87 ± 8.63 pg/ml, $p < 0.001$) (Fig. 1). Furthermore, higher levels of depression ($r = 0.53$, $p < 0.002$) and perceived stress ($r = 0.73$, $p < 0.001$) were significantly correlated with higher levels of plasma NGF (Fig. 2). Caregivers and controls did not differ significantly on health-related behaviors or plasma albumin levels (as a marker for general nutritional status), and there were no systematic group differences in medication use (data not shown).

4. Discussion

Dementia caregiving, a long-term stressor that can provoke significant dysregulation of cellular immunity and neuroendocrine homeostasis, is associated with a number of health risks, including mortality (Kiecolt-Glaser and Glaser, 1999; Kiecolt-Glaser et al., 1991, 1995, 1996; Schulz and Beach, 1999). In this study, we show that elderly caregivers experiencing chronic stress associated with caregiving (reflected in high perceived stress and depression scores) have elevated concentrations of plasma NGF. This finding extends published observations that the acute stressor, parachute jumping, can increase the levels of blood NGF in young adults (Aloe et al., 1994), and demonstrates that both types of psychological stressors can enhance the synthesis and/or release of NGF, regardless of the subject's age.

A previous report showed a relationship between NGF blood levels and stress, but not depression (Alleva et al., 1996a,b). Although we believe that NGF differences in this study were related to the chronic stress of caregiving, we cannot separate the impact of perceived stress from that of depressive symptoms on NGF blood levels in this study. Further, our depressed caregivers are representative of dementia caregivers in that depressive symptoms and perceived stress are typical; indeed, depressive disorders may occur in as many as 81% of dementia caregivers over the course of caregiving (Schulz et al., 1990). In fact, it is very difficult to separate the conceptual constructs of chronic stress and depressive symptoms because they have so much in common; moreover, they do not appear to have distinctly different immune or neuroendocrine relationships (Herbert and Cohen, 1993a,b).

Additional support linking stress to NGF modulation are data which show that aggression in male mice, as well as submission and defeat, was accompanied by a massive release of NGF into the blood stream (Lakshmanan, 1986, 1987; Aloe et al., 1986), and increased NGF mRNA and protein in the hypothalamus (Spillantini et al., 1989; Aloe

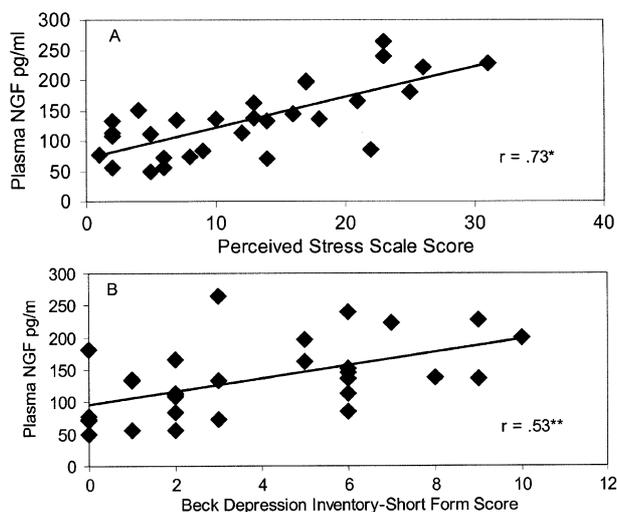


Fig. 2. Correlation between Perceived Stress Scale scores and plasma NGF (A) and Beck Depression Inventory-Short Form scores and plasma NGF (B). $N = 30$. Plasma NGF was measured as described in the Materials and Methods. * $p < 0.001$, ** $p < 0.002$.

et al., 1990). Possible sources of NGF to account for these increases include salivary glands, mast cells, basophils, and lymphocytes (Aloe et al., 1997; Leon et al., 1994).

It has been speculated that anxiogenic stimuli are the most likely psychological/biochemical substrate(s) underlying NGF synthesis and/or release into the blood (Levi-Montalcini et al., 1995; Aloe et al., 1990). In a previous study, NGF increased in the blood of first-time parachutists the night before the jump (Aloe et al., 1994). It has also been shown that anxiety during alcohol or heroin withdrawal was correlated with a rise of NGF in the blood (Aloe et al., 1996). Lack of control in a conflict situation, such as male mice experiencing repeated defeat and submission (Maestripietri et al., 1990) or lactating mothers defending their litters from unfamiliar male intruders (Alleva et al., 1996a,b), has been thought to be one of the variables that modulate the synthesis and/or release of NGF during stressful events (Aloe et al., 1986). This study suggests that exposure to the chronic stress of caregiving for a spouse with dementia can alter the concentrations of circulating NGF and that different types of psychological stress, acute and chronic, can induce changes in the production of NGF regardless of age.

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