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Expression of Neurotrophic Factors in Neonatal Rats After Peripheral Inflammation

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Abstract: **Neonatal peripheral inflammatory insult might result in the alteration of neuronal development in the nociceptive circuit. During early postnatal period, neurotrophins play important roles in neural development and sensory nerve innervation in the central and peripheral nervous systems. In this study, we investigated mRNA expression for neurotrophic factors and their receptors in the dorsal root ganglia of rat pups during postnatal life after peripheral inflammation induced by injection of complete Freund's adjuvant (CFA) into hind paw on postnatal day 1. Our results showed that mRNA expression levels of α–calcitonin gene-related peptides, tropomyosin-related kinase–A (trkA), p75 neurotrophin receptor ($p75^{NTR}$), and brain-derived neurotrophic factor (BDNF) elevated significantly after CFA treatment. Such an increase began 1 day after CFA treatment and lasted 2 to 3 days for trkA, $p75^{NTR}$, and BDNF. In contrast, there was no change in mRNA expression levels for neurotrophin-4/5, β–nerve growth factor (β-NGF), trkB, glial cell line–derived neurotrophin factor, and receptor protein tyrosine kinase protein. Our study demonstrated that neonatal peripheral inflammatory insult might result in molecular changes of neurotrophic factors, particularly in NGF receptors and BDNF, in the process of neuronal development and plasticity in primary afferents during early neonatal period.**

Perspective: ***Neonatal peripheral inflammation model has been used for the exploration of neuropathic pain mechanism for years. This work provided further detailed information about possible neurotransmitters and peptides involved in this process. This might also lead to future clinical application.***

Key words: *Neurotrophin, primary afferent, dorsal root ganglia, neonatal peripheral inflammation, quantitative RT-PCR.*

Noxious stimulation is normally absent or very rare in the neonate.[24,40] Interestingly, evidence shows that painful experiences in infants might alter their response to future painful situations.[43,44] It has been speculated that pain and nociceptive signaling in the newborn contribute to an alteration of normal developmental processes. Recent studies have indicated that peripheral inflammation experienced during the neonatal period has long-standing consequences on nociceptive neuronal circuits.[26,40,46] In adult rats that have experienced neonatal peripheral inflammation, a dramatic increase of nonmyelinated nervous fiber density in the primary afferent terminals of the spinal cord ipsilateral to the insult was well-correlated to subsequent behavioral and electrophysiologic responses to sensory stimuli.[35,42] As a possible molecular mechanism behind this observation, Ling et al[27] have demonstrated a dy-

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namic change in the pattern and distribution of calcitonin gene-related peptide (CGRP) terminals in various regions of the dorsal horn after neonatal peripheral inflammation during the critical neonatal development period in rats. However, other molecules might possibly be involved.

Nerve growth factor (NGF) and other neurotrophic factors and their receptors, including brain-derived neurotrophic factor (BDNF), glial cell line–derived neurotrophin factor (GDNF), neurotrophin-3 (NT-3), and NT-4/5, are known to play important roles in regulating neural development and neuronal survival in both the peripheral and the central nervous systems during development and in maturity. Peripheral injury results in the release of growth factors including neurotrophic factors and cytokines with inflammation; the release might be especially strong in the neonatal stage.[13] Because sensory neurons are critically dependent on neurotrophic factors in neonates,[1,12] the released neurotrophic factors accompanying peripheral inflammation might affect or alter the normal development of sensory innervation in the central or peripheral nervous systems.

In addition to neurotrophins, CGRP and substance P (SP) are postulated to be involved in nociceptive circuit plasticity at the level of the spinal cord. Expression of CGRP and SP mRNA in primary afferent neurons is generally reported to increase in response to peripheral inflammation.[15] The distribution of CGRP in primary afferent neurons indicates co-localization with tropomyosin-related kinase (trk) protein. Virtually all trkA-immunoreactive cells express CGRP immunoreactivity.[3,37] On the other hand, changes in density and distribution of CGRP containing C- and Aδ-primary afferents after spinal cord injury can be manipulated by changes in endogenous levels of NGF.[7] In contrast, neonatal treatment with anti-NGF reduces the proportion of neurons in the dorsal root ganglia (DRGs) that express CGRP during the critical neonatal period in rats.[45] Therefore, although the functional interaction between CGRP and NGF remains to be clarified, current data suggest that they are both involved in the process of neuronal plasticity.

We designed this study to further explore the possible involvement of neurotrophins and their receptors during the early postnatal period after peripheral inflammatory insult. Changes in the mRNA expression of CGRP and neurotrophic factors and of their receptors in the DRGs of rat pups during the early postnatal period after unilateral injection of complete Freund's adjuvant (CFA) into hind paws on postnatal day 1 (P1) were examined.

Materials and Methods

Animals and Inducement of Inflammation

This study was approved by the Animal Research Facility of the Cathay Medical Research Institute, Cathay General Hospital. Timed-pregnancy Sprague-Dawley rats were monitored to determine the time of birth of rat pup litters. To induce peripheral inflammation in the experimental group, male rat pups were injected with 25 μL of CFA (Sigma, St Louis, MO) (2:1, CFA: saline) unilaterally into the left hind paw on postnatal day 1 (P1). For the control group, the same volume of saline was injected into the hind paw on the same side on the same day.

Table 1. Primers for Identification by PCR

Gene	*GeneBank No.*	*Primers*
α-CGRP	M11597	5′-aagttctccccttttcctggttgtca-3′ 5′-tggtgggcacaaagttgtccttcac-3′
β-NGF	XM_227525	5′-acctcttcggacactctgga-3′ 5′-gtccgtggctgtggtcttat-3′
BDNF	NM_012513	5′-aggacgcggacttgtacact-3′ 5′-aagttgtgcgcaaatgactg-3′
GDNF	AF497634	5′-gactccaatatgcccgaaga-3′ 5′-cttcacaggaaccgctacaa-3′
NT-4/5	S69323	5′-cactggctctcagaatgcaa-3′ 5′-gtagagcagtcgagccatcc-3′
$p75^{NTR}$	NM_012610	5′-ctgccaggacaaacagaaca-3′ 5′-tatccccgttgagcagtttc-3′
TrkA	NM_021589	5′-tacctagccagcctgcactt-3′ 5′-gggttgctttccataggtga-3′
TrkB	M55293	5′-ttagcctcgtcaggtgcttt-3′ 5′-ttttggtttcccttccactg-3′
Ret	AJ299017	5′-aaacatcttggtggcagagg-3′ 5′-catcaggcggtacatttcct-3′

Tissue Collection

After CFA and saline injection, rat pups were killed on P1 (8 hours after injection), P2 (24 hours after injection), P3, P4, P6, P8, P15, and P22. For euthanasia, animals were administered an overdose of sodium pentobarbital injected intraperitoneally. The left (ipsilateral) or the right (contralateral) side of the fourth/fifth lumbar (L4/L5) DRG was removed under a surgical microscope. Tissues were frozen at −80°C until RNA isolation.

Isolation of RNA and Quantification

Total RNA was isolated by using the 3-*Zol* reagent (MDBio, Taipei, Taiwan) methods, and the RNA sample was treated with DNaseI (Promega, Madison, WI) to remove traces of genomic DNA. To assure optimal DNaseI activity, the buffer conditions in the RNA solution were adjusted accordingly. RNA absorbance at 260 nm was measured by using a spectrophotometer to obtain a yield in micrograms per microliter (μg/μL). Quantitative reverse transcription–polymerase chain reaction (RT-PCR) was performed with equal amounts (1 μg) of total RNA from the L4/L5 DRGs of individual animals on both the ipsilateral and the contralateral sides. cDNA was synthesized and amplified by using a Titanium One Step RT-PCR Kit (BD Biosciences Clonetech, Palo Alto, CA). Primers directed toward α-CGRP, β-NGF, trkA, $p75^{NTR}$, BDNF, trkB, NT-4/5, GDNF, and receptor protein tyrosine kinase protein (Ret) were used for amplification (Table 1). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used as an internal control. For quantitative analysis, it is necessary to determine the linear correlation