TENS-like Stimulation Downregulates Inflammatory Cytokines in a PC-12 Cell Line

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Abstract

Objectives: The purpose of this study was to evaluate the effects of transcutaneous electrical nerve stimulation (TENS)–like stimulation on the expression of the proinflammatory cytokines tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), and IL-6 in PC-12 cells, which are commonly used as neuronal cell models.

Methods: Nerve growth factor–differentiated PC-12 cells were exposed to electrical stimulation for 15 minutes at 1 mA, 200 μs, and 100 Hz. Cell lysate from stimulated and control cells was assayed for TNF-α, IL-1β, and IL-6. In 6 trials, cells were preincubated with the L-type ion channel blocker nicardipine. Cultured cells were also incubated with Alexa Fluor 488 and visualized by fluorescence microscopy to determine the nuclear vs cytoplasmic distribution of the p65 sub-unit of NF-κB

Results: Compared with control (unstimulated) cells, the stimulated cells had a downregulation of the assayed cytokines. However, preincubation with the L-type ion channel blocker nicardipine blocked this effect of stimulation. Additionally, it was noted that TENS-like stimulation promoted a relative sequestration of the p65 subunit of NF-κB in the cytoplasm vs the nucleus.

Conclusions: It appears that in this cell line and with these stimulation parameters, TENS-like stimulation attenuated the expression of the assayed proinflammatory cytokines, in part by promoting the relative sequestration of the p65 subunit of NF-κB in the cytoplasm, and that voltage-dependent calcium channels have a role in the cascade of events initiated by the TENS-like stimulation. (J Manipulative Physiol Ther 2017;40:381-386)

Key Indexing Terms: *Transcutaneous Electrical Nerve Stimulation; Cytokines*

Introduction

Transcutaneous electrical nerve stimulation (TENS) is a noninvasive, nonpharmacologic treatment method that is widely used for the management of musculoskeletal pain. TENS can be self-administered, and significant adverse events are uncommon; for example, see Magis et al[1] and Yates et al.[2] TENS has been reported to relieve myofascial neck pain,[3] reduce tenderness and pain at rest in rheumatoid arthritis of the hand,[4] and relieve pain in osteoarthritis of the knee.[5] On the other hand, a recent review by Khadilkar et al[6] did not establish the efficacy of TENS in relieving chronic low back pain. Nonetheless, evidence supports the use of TENS in different populations, such as the elderly, pregnant women, and patients with nonradicular low back pain.[7-9] It is apparent that more qualitative research needs to be done in order to establish the types of pain that can be managed with TENS. Nevertheless, the weight of evidence indicates that TENS is beneficial at least for certain types of pain and in certain populations.

The cellular mechanisms of pain relief associated with TENS remain largely unresolved, and this may present a barrier to more effective design and interpretation of clinical studies. One proposed mechanism is opioid-mediated activation of descending inhibitory pathways involving the rostral ventral medulla, with antihyperalgesia by the activation of both μ- and δ-opioid receptors in the rostral ventral medulla.[10] In a rat model, TENS has been reported to control, by inhibition of ERK1/2-COX-2 pathways, inflammatory pain induced with complete Freund's adjuvant.[11] In another rat model for skin and muscle incision and retraction, TENS was administered via the ipsilateral dorsal rami of L1-L6 and inhibited upregulation of the proinflammatory cytokines tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), IL-6, and substance P.[12] The physiologic effects of TENS beyond pain reduction remain largely unexplored, although in a recent study in a rat model of spinal cord compression, it was reported that TENS augmented spinal cord blood flow for periods of up to 15 minutes of stimulation.[13] The physiologic mechanisms by which TENS exerted this effect were not investigated. However, one candidate mechanism would be upregulation of proinflammatory cytokines.

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Hence, the present study was designed to determine whether, in neuronal tissue culture, levels of proinflammatory cytokines were modulated by TENS-like stimulation and to elucidate the subcellular events associated with any induced changes. Here, we refer to the stimulation used as "TENS-like" because in cell culture, of course, the stimulation is not transcutaneous and it is directed at cells rather than whole nerves. Nonetheless, the actual parameters of stimulation used are identical to those used previously in studies of authentic TENS (for example, see Budgell et al). The cell line used was nerve growth factor (NGF)–differentiated PC-12 cells, which have biogenesis of neurotransmitters, acquisition of electrical excitability, and growth of neurites. Thus, this cell line is considered a good model for the study of neuronal function.[14,15]

The purpose of this study was to evaluate the effects of TENS-like stimulation on the expression of the proinflammatory cytokines TNF-α, IL-1β, and IL-6 in PC-12 cells, which are commonly used as a neuronal cell model.

Methods

Cell Culture

PC-12 cells (ATCC, Rockville, MD) were cultured in RPMI1640 supplemented with 10% heat-inactivated horse serum, 5% fetal bovine serum, and 1% penicillin-streptomycin (Life Technologies, Burlington, ON, Canada). Cells were incubated at 37°C under 5% CO_2. Induction of the neuronal phenotype was achieved by treating the cells with 100 ng/mL NGF (Cedarlane, Burlington, ON, Canada) twice, 2 and 4 days before experimentation.

Electrical Stimulation and Ion Channel Blocking

In 7 trials, cell cultures were electrically stimulated via 2 copper electrodes (1.0 mm diameter) placed on opposite sides of the culture dishes. Current was delivered for 15 minutes at 1 mA, 200 μs, and 100 Hz. Control cultures were exposed to the copper electrodes for 15 minutes but received no current.

In an additional 6 trials, cell cultures were exposed to the L-type ion channel blocker nicardipine (Sigma-Aldrich, Oakville, ON, Canada), diluted in dimethyl sulfoxide (Sigma-Aldrich) to a final concentration of 10 μM in fresh growth medium for 30 minutes before TENS-like stimulation.

Cell Lysate Preparation for Protein Analysis

Cells were washed twice with phosphate buffered saline (PBS), scraped in 600 μL PBS and spun down at 2500 rpm for 5 minutes at 4°C. The supernatant was removed and the pellet was lysed in 200 μL radioimmunoprecipitation buffer (25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate) supplemented with 1 mM phenylmethylsulfonyl fluoride, 2 μg/mL peptstatin, and 2 μg/mL leupeptin (Sigma-Aldrich). Cell lysate was spun down at 14,000 rpm for 15 minutes at 4°C and the protein concentration was measured.

Dotblot Analysis

TNF-α, IL-1β, IL-6, and NF-κB were detected by dotblot analysis. Protein (100 μg/mL) was adsorbed to a nitrocellulose membrane. After blocking, the membrane was incubated with rabbit antirat TNF-α, IL-1β, and IL-6 (Life Technologies), rabbit antirat NF-κB p65 (eBioscience, San Diego, CA), and rabbit antirat glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Cedarlane). Then the membranes were washed and incubated with alkaline phosphatase goat antirabbit immunoglobulin G antibody and detected by 5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium colorimetric assay (Life Technologies). Integrated densities were obtained using ImageJ 1.48v (National Institutes of Health, Bethesda, MD), and data were normalized to GAPDH and controls.

Immunofluorescence Microscopy

PC-12 cells were plated on glass coverslips under the same culture conditions as described earlier. After electrical stimulation, the cells were washed twice in PBS, fixed with 4% formalin (Sigma-Aldrich) at room temperature for 10 minutes, and washed with PBS. Coverslips then were moved to humidity, covered in 4°C methanol for 5 minutes and washed with PBS. Cells were blocked (0.5% bovine serum albumin in PBS) and incubated with p65, after which they were incubated with Alexa Fluor 488 goat antirabbit conjugated (Life Technologies). After washing, the cells were mounted on glass slides with DAPI mounting media and were visualized by fluorescence microscopy (Axio Scope A1, Zeiss, Göttingen, Germany). P65 subcellular distributions in 10 separate samples from both control and TENS-treated slides were recorded.

Data Analysis

Statistical analysis was conducted using IBM SSPS (IBM Corp., Armonk, NY). Levels of cytokines in cell lysate from control vs TENS-exposed cells were compared using the 2-tailed unpaired t test, with a threshold of significance set at $P = .05$. This was done both for cells with and without preincubation with nicardipine.

Cytoplasmic vs nuclear distributions of the p65 subunit of NF-κB in control vs TENS-exposed cells were compared using the χ^2 test with the threshold of significance set at $P = .05$.

Results

As shown in Figure 1, in the lysate of control vs TENS-treated cells, the concentrations (mean ± standard