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Age Differences in the Time Course and Magnitude of Changes in Circulating Neuropeptides After Pain Evocation in Humans

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Abstract: **This study tested the hypothesis that older adults would have a stronger response for substance P (facilitatory) but weaker response to β-endorphin (inhibitory), in magnitude as well as time course. Eight younger and 9 older adults underwent 3 experimental sessions using well validated laboratory pain models: cold pressor task, contact heat pain, and a nonpainful control. Blood was collected through an indwelling catheter at baseline and 3, 15, 30, 45, and 60 minutes after stimuli administration. Older adults had higher baseline levels of both neuropeptides suggesting increased peripheral activity compared with younger adults. After the cold pressor task, older adults demonstrated a quick and strong release of substance P with dramatic recovery, whereas young adults maintained a constant low-grade response. Unlike substance P, β-endorphin increased between 3 and 15 minutes for both groups with the upsurge substantially higher for older adults. After heat pain, younger adults had an immediate surge in circulating substance P and β-endorphin that was more pronounced than among older adults. However, levels of substance P for younger adults slowly tapered whereas they continued to climb for the older adults through 30 minutes. β-endorphin peaked at 30 minutes for both groups and returned to baseline. No changes were observed during the nonpainful control session.**

***Perspective:** Older adults had higher baseline levels of substance P and β-endorphin suggesting increased peripheral activity compared with younger adults. After pain evocation, older adults demonstrated a more intense early response for both neuropeptides suggesting peripheral mechanisms involved in the response to pain may change with age.*

Key words: *Aging, pain, neuropeptides, biomarkers, substance P, β-endorphin.*

Aging is a biological process associated with changes in the structure and functioning of multiple biological systems.[56] This includes systems involved in pain processing and may explain why older adults are at increased risk to develop more frequent and prolonged pain, as well as chronic pain at multiple sites.[13,43] Evidence suggests contributing factors include dysfunctional pain inhibitory and excitatory mechanisms[33] and immune dysregulation (ie, inflammaging).[16]

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In an earlier report, we presented data showing age differences in cytokine cascades induced by standardized painful experimental stimuli.[8] In this report, we present data on substance P and β-endorphin, commonly reported pain-related neuropeptides. Neuropeptides are a set of diverse intercellular signaling molecules that are contained within and released from a range of neurons across the central and peripheral nervous system.[55] These proteins differ from a range of small-molecule neurotransmitters (ie, norepinephrine, dopamine, serotonin, gamma-aminobutyric acid, glutamate, etc) in size and are not able to cross the blood-brain barrier.[44] Consequently, the peripheral activity of neuropeptides in relation to pain in aging is of interest.

Substance P is a neuropeptide from the tachykinin family that is an excitatory neurotransmitter involved in pain transmission.[22,40,42] Upon tissue damage and/or pain evocation, C-fibers release substance P through neurokinin-1 receptor activation in afferent sensory neurons at the dorsal horn of the spinal cord. At the peripheral level, substance P directly stimulates nociceptors and facilitates increased nociceptive input to the spinal cord.[7,34] In addition, it plays an important role in peripheral inflammatory response acutely (redness, heat, swelling after injury) as well as chronically (eg, increased expression in arthritic joints).[7,52]

In contrast, β-endorphins are inhibitory neuropeptides produced by pituitary gland and released into peripheral circulation during stressful and painful events. β-Endorphins are also produced peripherally via organ and tissue cells, including the immune system cells. A principal role of circulating β-endorphin after painful and stressful events is to bind to opioid receptors pre- as well as postsynaptically, which inhibits neuronal firing of peripheral somatosensory fibers.[36,51] In the animal literature, electric foot shocks, restraint, or swim stress increases β-endorphin as well as substance P plasma concentrations.[1,11,27,63,64]

To date, there are no studies that have tested for age differences in basal and pain-evoked circulating levels of these pain-related neuropeptides in healthy adults. Thus, the overall goal of the present investigation was to test for differences between healthy younger and older individuals on the time course and magnitude of changes of substance P and β-endorphin in plasma. This study was performed using well validated experimental pain models: 1) the cold pressor task (CPT), 2) contact heat, and 3) a nonpainful thermal control administered in separate sessions. Stimuli levels were individualized so that each subject experienced a standardized level of pain. Therefore, time course and changes observed on the biological biomarkers occurred under the same amount of pain. The primary hypotheses for this study was that older adults would have a stronger response for substance P (facilitatory) but a weaker response to β-endorphin (inhibitory), in magnitude as well as time course. We also hypothesized that no changes would occur during the nonpainful control session.

Methods

Participants

Study participants were 8 younger healthy adults (4 male, 4 female) with a mean age of 21.4 (SD = 5.8) years and 9 older healthy adults (5 male, 4 female) with a mean age of 68.1 (SD = 6.0) years. All 4 older women reported postmenopausal status and the 4 younger women reported having regular menstrual cycles and were not taking birth control pills. There were no differences in body mass index between the younger sample (24.6, SD = 3.0) and older sample (24.8, SD = 2.5). Potential participants were screened via the phone and again in person during the first visit. Study rule-outs were: current use of narcotics or tobacco products, chronic or current use of analgesics, uncontrolled hypertension, other serious systemic disease (eg, diabetes, thyroid problems, etc), neurological problems with significant changes in somatosensory and pain perception, serious psychiatric conditions (eg, schizophrenia, bipolar disorder), currently receiving treatment for chronic pain (eg, low back pain, postherpetic neuralgia) or any ongoing pain problem (headaches, arthritis, injury-related pain, etc), pregnant or trying to get pregnant (for younger women). All participants provided informed consent after details of the study were explained. The University of Florida Institutional Review Board approved the study.

When enrolled, participants were instructed a day before each scheduled session to avoid any heavy, vigorous exercise from 12 AM before each session, to have breakfast no later than 1 hour before the scheduled experimental session, and to avoid dairy products, foods with high sugar, acidity, or fat content, drinks with caffeine content, or any alcoholic beverage. In addition, participants were asked to avoid staying up late the night before the experiments and to try to go to bed at their usual bedtime. All experimental sessions were scheduled at the same late morning time for all participants.

Experimental Sessions

Sessions were all conducted in standardized, quiet rooms at the University of Florida Clinical and Translational Science Institute Clinical Research Clinic. Staff nurses supervised urine collection for pregnancy testing for female participants. Sessions began with participants sitting in a reclining chair and resting for 5 minutes. Two blood pressure readings separated by 5 minutes were taken using a Datascope Accutorr Plus blood pressure monitor (Datascope Inc, Mahwah, NJ). If there was a change in blood pressure > 5%, participants were instructed to rest for an additional 5 minutes and a third blood pressure measurement was taken. Staff nurses inserted an indwelling catheter in the left forearm and participants rested for an additional 15 minutes. Testing began with collection of a baseline blood sample followed by exposure to the painful stimuli for that session (ie, CPT, contact heat, warm control). Additional samples of blood were collected at 3, 15, 30, 45, 60, and 90 minutes. The order of experimental sessions was counterbalanced.