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Age differences in the time-course and magnitude of changes in circulating neuropeptides following 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), both in magnitude and time-course. Eight younger and 9 older adults underwent 3 experimental sessions using well-validated laboratory pain models: cold pressor task (CPT), contact heat pain (HP), and a non-painful control. Blood was collected through an indwelling catheter at baseline and 3, 15, 30, 45, and 60-minutes post-stimuli administration. Older adults had higher baseline levels of both neuropeptides suggesting increased peripheral activity compared to younger adults. Following CPT, 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

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between 3 and 15 minutes for both groups with the upsurge substantially higher for older adults. Following HP, 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 non-painful control.

Keywords

aging; pain; neuropeptides; biomarkers; substance P; β -endorphin

Introduction

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 (i.e., inflammaging) (16).

In an earlier paper, 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 (i.e., norepinephrine, dopamine, serotonin, GABA, 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 neurokinin1 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 both acutely (redness, heat, swelling after injury) and chronically (e.g., increased expression in arthritic joints) (7,53).

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 following painful and stressful events is to bind to opioid receptors both pre- and post-synaptically; which inhibits neuronal firing of peripheral somatosensory fibers (36,52). In the animal literature, electric foot shocks, restraint or swim stress increases both BE and SP plasma concentrations (1,11,63,64).

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: (a) the cold pressor task, (b) contact heat and (c) a non-painful 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), both in magnitude and time course. We also hypothesized that no changes would occur during the non-painful control session.

Materials and Methods

Participants

Study participants were 8 younger healthy adults (4 males, 4 females) with a mean age of 21.4 (SD = 5.8) and 9 older healthy adults (5 males, 4 females) with a mean age of 68.1 (SD = 6.0). All four older females reported post-menopausal status and the four younger females 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 over 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 (e.g. diabetes, thyroid problems, etc.), neurological problems with significant changes in somatosensory and pain perception, serious psychiatric conditions (e.g., schizophrenia, bipolar disorder), currently receiving treatment for chronic pain (e.g., low-back pain, postherpetic neuralgia) or any ongoing pain problem (headaches, arthritis, injury-related pain, etc.), pregnant or trying to get pregnant (for younger females). All participants provided informed consent once details of the study were explained. The University of Florida Institutional Review Board approved the study.

Once enrolled, participants were instructed a day prior to each scheduled session to avoid any heavy, vigorous exercise from 12 AM prior to 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, 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 (CRC). 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 greater than 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 (i.e., cold pressor task, 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.

Cold Pressor Task (CPT)—Participants immersed their foot in 8°C (males) or 10°C (females) water, which was constantly recirculating to prevent local warming or cooling around the submerged foot. The cold stimulus was administered in four 60-second trials with a 60-second rest/withdrawal period between trials. Our earlier work has shown that males and females report equivalent levels of pain at these temperatures. The water level was set at a height of 7 cm in order to keep the stimulated area consistent. Participants provided ratings of cold pain during each of four 60-second trials at 15-sec, 30-sec, and 45-sec for a total of 12 pain ratings. Testing temperatures were adjusted between trials if needed to obtain targeted pain ratings of between 40–50 (VAS: 0=no pain and 100=intolerable pain). For example, if participants provided a peak VAS pain rating above 50 during a CPT trial, the temperature was adjusted upwards for the next trial.

Contact Heat Pain (HP)—Similar to the CPT, painful heat stimuli were administered in four 60-second trials with a 60-second rest/withdrawal period between trials. Starting temperatures of 48°C (males) and 47°C (females) were used and adjusted to reach the targeted pain ratings of 40–50 VAS as needed. The rating procedure and adjustments of thermode temperature followed the same pattern as described above for CPT. The Medoc Pathway Thermal Sensory Analyzer (Ramat Yishai, Israel) was used for the thermal stimulation using a 16×16mm ATS thermode.

Non-painful Control—Application of a non-painful warm stimulus (32–34°C) was used as the control session, which was identical to the CPT and HP sessions except for the stimulation temperature used. The warm stimulus was similarly administered in four trials of about 60 seconds each with a 60-second rest/withdrawal period between trials. Participants were asked to rate the warm stimulus using a VAS (0=No pain sensation and 100=Maximal pain sensation). No participants rated any of the control trials as painful. The Pathway (Medoc, USA) was used for the thermal stimulation using a 16×16mm ATS thermode.

Plasma Collection and Soluble Mediator Analysis

Established protocols were followed for blood collection using heparin-coated tubes. All samples were immediately placed on ice and transported to the CRC laboratory where they were centrifuged at 12,000 rpm for 15 min. All plasma was stored at –80°C until assays could be performed. Plasma soluble mediators were qualitatively and quantitatively evaluated utilizing the MILLIPLEX XMAP (Millipore, Billerica, MA). Briefly, plasma and antibody-coated beads were added to 96-well primed filter plate and allowed to incubate overnight at 4°C. Following three washes, biotinylated detection antibodies were allowed to

incubate for 1 hour at room temperature, after which streptavidin (SAV)-phycoerythrin was allowed to incubate for 30 min. Following three washes, beads were resuspended in sheath fluid and reactivity acquired using a Luminex 200 IS system (Luminex, Austin, Tx). Concentrations in pg/ml were determined using a standard curve, 5-parameter logistics and Milliplex analyst (Viagene). A panel of pro- and anti-inflammatory cytokines was also processed at this time but are not part of this manuscript.

Statistical methods

The primary aim of this study was to test the hypothesis that older adults would have a stronger substance P (facilitatory) response but a weaker β -endorphin (inhibitory) response to painful cold and heat stimulation. To accomplish this, age differences in each neuropeptide profile for both CPT and HP sessions were tested using repeated-measures ANOVA.

The dependent variables for this study were circulating substance P and β -endorphin concentrations expressed in pg/ml, adjusted for baseline levels calculated as *post-stimulation – baseline*. Comparisons were made between baseline and 5 post-stimulation time-points; 3-min, 15-min, 30-min, 45-min, and 60-min. Circulating levels had generally returned to baseline by 90-min. The primary independent variable was “age” and tested for differences between the younger and older groups in the magnitude of the neuropeptide response. The “age $\times$ time” interaction effect was also examined and tested for age differences in the time-course of neuropeptide expression. Sex was used as a covariate because of known sex differences in pain but was not significant on any set of analyses (15). When interaction effects were significant, pair-wise comparisons were made only across each time-point and not between time-points to reduce the number of statistical tests performed. Mauchly’s Test of Sphericity assessed whether the assumption of sphericity had been violated (37); and if so, we applied the Greenhouse-Geisser correction (21). Using repeated-measures ANOVA, we tested differences in plasma concentrations at baseline, 3, 15, 30, 45, and 60 minutes for the warm session. The primary independent variable of interest for this hypothesis was “time”, which tested the magnitude of the overall neuropeptide response. Sex and age were used as covariates in this set of analyses. Baseline concentrations were averaged across the three sessions and ANOVA was used to test for differences across age groups.

Results

The mean pain rating for the experimental sessions, which were calculated by averaging across the 12 pain ratings taken during each of the four administrations of CPT or HP using a 0–100 numerical rating scale, was 43.6 (SD = 19.7) for CPT and 50.2 (SD = 19.4) for HP. A repeated-measures ANOVA (session $\times$ age) indicated that the pain ratings were not significantly different between the sessions ($p = .17$) or between the age groups ($p = .80$). Consequently, all participants experienced equivalent levels of pain. The mean temperatures administered across the 4 trials for each session did not differ between the age groups (CPT, $p = .41$; HP, $p = .51$). The physiological response to both cold and heat pain testing was initially evaluated by systolic (SBP) and diastolic (DBP) blood pressure at baseline, stimuli induction and at discharge (Table 1). The mean changes for the CPT session were 7.7 (SD =

11.8) for SBP and 4.4 (SD = 13.4) for DBP, while for the HP session, the mean change was 5.6 (SD = 7.8) for SBP and 3.1 (SD = 7.2) for DBP. Repeated measures ANOVA indicated that there were no differences between sessions ($p = .20$) or as a function of age ($p = .51$). As a control, the plasma levels of substance P and β -endorphin in the absence of pain testing were first evaluated for up to 90 minutes. Importantly, neither of the two neuropeptides was significantly elevated compared to baseline in the absence of pain application.

Baseline levels

At baseline, both substance P and β -endorphin differed as a function of age [$F(1, 14) = 436.920$, $p < .001$ and $F(1, 14) = 393.887$, $p < .001$ respectively] in that older adults had higher circulating levels than younger adults. Baseline for each session is presented in Table 2 (substance P) and Table 3 (β -endorphin) displayed in Figure. 1.

Cold Pressor Task

Mean and standard deviation for change in plasma concentrations for substance P and β -endorphin are shown in Table 2 and 3 respectively. The overall effect of age for both substance P [$F(1, 14) = 26.030$, $p < .001$], and β -endorphin [$F(1, 14) = 8.728$, $p = .010$] were statistically significant. However, the age $\times$ time interaction was significant for both neuropeptides [substance P; $F(1.684, 23.579) = 86.385$, $p < .001$ and β -endorphin; $F(1.010, 14.134) = 29.770$, $p < .001$] indicating that examining age differences at each time point provided the best interpretation of the results.

Following the CPT, older adults demonstrated a significantly greater release of substance P than the younger group, which returned to near baseline levels after 30 minutes (Figure 2a). In contrast, young adults maintained a small change in substance P level (~ 5 pg/ml from baseline) through 60 minutes of recovery time. During recovery, the younger group demonstrated greater levels of substance P compared to baseline at 30- and 45-min after CPT, and the difference disappeared by 60-min (Figure 2a). Unlike substance P, β -endorphin demonstrated robust increases between 3 and 15 minutes for both groups and returned to near baseline by 30 minutes (Figure. 2b). For β -endorphin, there were no group differences at 3-min, but at 15-min after CPT, greater increase in plasma β -endorphin from baseline were seen for the older group compared to the younger group. During recovery, the groups demonstrated similar levels compared to baseline (Figure 2b).

Contact heat pain

Mean and standard deviation for change in plasma concentrations for substance P and β -endorphin are shown in Table 2 and 3 respectively. The overall effect of age for both substance P [$F(1, 14) = 26.618$, $p < .001$] and β -endorphin [$F(1, 14) = 55.130$, $p < .001$] were statistically significant. Similarly to the CPT, the age $\times$ time interaction was also significant for both neuropeptides [substance P; $F(1.222, 17.112) = 33.123$, $p < .001$ and β -endorphin; $F(1.172, 16.408) = 13.971$, $p = .001$] indicating that examining age differences at each time point provided the best interpretation of the results.

After HP, across both groups, induction of plasma substance P and β -endorphin levels occurred by 3 minutes (Figure. 3a and 3b) and increased to a peak at 30 minutes before

resolution baseline at 45 minutes. The younger group showed a significantly greater increase in plasma level of both substance P and β -endorphin from baseline than the older group at 3- and 15-min after HP, with the older group demonstrated greater change at 30-min for substance P. During recovery (45- and 60-min after HP), both groups returned to values close to baseline, with the younger group presenting with slightly greater levels of substance P compared to baseline and older group a significantly lower level of substance P than baseline. Consequently, there was significant difference in the recovery level of both neuropeptides with younger having higher concentration compared to the older group.

The associations between baseline levels of substance P and β -endorphin for both age groups at each session were high ($r < .95$, $p < .001$). The correlations between baseline values and changes in plasma concentrations at all time points for the CPT model indicated the associations were linear for both substance P and β -endorphin ($r = 1.0$, $p < .001$). Correlations between baseline levels and changes in plasma concentrations for the HP model are varied and are presented in Table 4. In the younger sample, change in biomarker concentrations following HP stimulation were positively associated with baseline values for both biomarkers at elevated time points (through 30-min). Residual values (45-min and later) were not associated with baseline levels. Similar to the younger group, peak concentrations in the older group were also positively associated with baseline levels. However, negative associations were observed in the older group following recovery with values below baseline. Therefore, higher baseline levels resulted in lower concentrations during the recovery period.

Discussion

While recent research has focused on understanding central changes during chronic pain conditions, peripheral mechanisms have not received the same attention (50). However, peripheral mechanisms play an important role in pain processing (20). Primary afferent nociceptors are sensitized by release of neuropeptides upon peripheral injury or inflammation (peripheral sensitization). In turn, the stimulus threshold is lowered resulting in a prolonged and enhanced stimulation response (26), which can result in sensitization of spinal cord neurons (32).

A novel component to this study was the use of two different human pain models. Cold pain using the CPT produces sensations of burning or deep aching pain (31,62), likely involving cutaneous vein nociceptors (17,26) and both A δ and C-fibers (9,10). Cold pain also involves cardiovascular (56,57) and autonomic (35) responses that interact with pain processing (47). In contrast, contact heat produces sensations described as prickly, throbbing, burning or swelling (24) and is associated with predominately C-fiber activity (38,60). While not the primary focus of this study, we did observe model differences, primarily that peak neuropeptide changes occurred later following heat pain than cold pain. Notably, cardiovascular responses were similar for both pain models despite the larger stimulated area in the CPT.

In a simpler context, Substance P (excitatory) and β -endorphin (inhibitory) would appear to work by providing a homeostatic balance of cellular and behavioral effects regulating the

perception of pain. The most well-established mechanism would involve the analgesia produced by β -endorphin through binding to mu-opioid receptors which results in inhibition of neuronal firing of nociceptive neurons, as well as with inhibition of substance P release (28,30,49,53). This binding and subsequent inhibition of substance P can occur at the peripheral sensory nerves synapse at dorsal root of the spinal cord (60) and at the peripheral end of these sensory nerve fibers and their terminals (23,54). Therefore, investigating age differences in circulating neuropeptides, particularly substance P and β -endorphin, may provide insight into the balance of these two mechanisms that could place older adults at increased risk to develop more frequent and prolonged pain and pain at multiple sites (13,25,43).

Age Differences in Circulating Neuropeptides

A paucity of human studies exist to determine age differences in substance P; though multiple studies have found decreased levels in the periphery of older animals (18). Twenty-four hour circulating β -endorphin patterns have been reported as similar across age, however, older adults have also demonstrated altered β -endorphin circadian rhythm patterns (46). Collectively, the current body of literature suggests that age differences in these circulating neuropeptides are either biomarker specific, highly variable, and/or dependent on other factors such as time of day and type of stimuli. For this study, we found substance P and β -endorphin in the late morning to be higher at baseline for older adults compared to younger adults. While our sample size was small, effect sizes were large across both neuropeptides.

Age Differences in Circulating Neuropeptide Levels after Pain Evocation

As mentioned, little information exists on potential age-related changes in circulating levels of substance P and β -endorphin during or after pain stimulation in humans. Substance P expression for younger rats with axonal injury was found to be similar to healthy older rats (4). One study found plasma β -endorphin levels to increase with CPT, but found no difference based on age (6). Another study reported no change in plasma β -endorphin with CPT, nor did they find age effects (5). Importantly, previous work has not comprehensively examined the time-course of circulating substance P and β -endorphin with pain evocation, as we have presented in this study.

Our findings suggest that neuropeptide changes are different based on age and nature of the painful stimulus. Following the CPT, older adults demonstrated a quick and robust release of substance P with a dramatic recovery, whereas young adults maintained a constant low-grade response. This finding is consistent with studies where β -endorphin, but not substance P was increased by acute stress in younger individuals (45,48).

Unlike substance P, β -endorphin demonstrated robust, rapid increases for both groups. Even so, the magnitude of this increase was substantially higher for older adults. In addition, the older group showed a dramatic change between 3 and 15 minutes post stimulation where an increase in expression of β -endorphin (43 to 87 pg/ml) was paired with a decrease in substance P (23 to 9 pg/ml) that was not seen for the younger participants. This observation was explored further by calculating the correlation between the changes in substance P and

β -endorphin for the older group, which was $r = -.95$ ($p < .001$). Although the direction of causality cannot be assumed, this indicates a greater increase in β -endorphin was strongly associated with a decrease in substance P. Taken together, it appears that older adults have a quicker and stronger increase in both facilitatory and inhibitory circulating neuropeptides than younger adults following cold pain evocation. The increased release of neuropeptides in older adults are also likely mechanisms to compensate for the age-related dysfunction in blood-brain-barrier permeability, SNS tone, and altered expression of neurotransmitters and receptors documented in old animals and human post mortem material (12,19,58). The CPT results support our hypotheses that older adults would have a stronger release of substance P (facilitatory), and based on the above reviewed literature, that β -endorphin would decrease expression of substance P. It is possible that this effect also occurred for younger adults; however the suppression occurred faster with substance P already attenuated by the 3-min time-point, whereas for the older participants, this process was slower.

Age differences in neuropeptide response also existed after HP. Contrary to our hypothesis, younger adults had an immediate surge in circulating substance P that was more pronounced than among older adults. Nonetheless, levels for younger adults slowly tapered for 30 minutes and eventually dropped to near baseline levels by 45-min. In contrast, older adults experienced an immediate surge in circulating substance P that continued to increase through 30 minutes post stimulation before returning to baseline. Substance P in the HP model was the one instance where peak levels were delayed for older adults (30-min, 28 pg/ml) compared to the younger sample (3-min, 26 pg/ml). The results for β -endorphin were consistent with our hypothesis in that older adults had a weaker initial response (14 to 30 pg/ml), but there was little difference by the 15-and 30-min time-points. It is also interesting to note that for the older adults, levels following recovery were below baseline and higher baseline values were paired with lower concentrations in the recovery period.

It is important to keep in mind that circulating levels substance P is necessary and play an important role in pain regulation through inflammation and plasma protein extravasation. Another consideration is the balance between circulating peripheral levels and available receptor sites. Although we documented increased levels of basal and stimulated substance P and β -endorphins in both groups, we cannot conclude on this finding alone that an age difference in plasma levels indicates greater physiological activity. Opiate receptors are reported to decline with advancing age (39). There is very limited data on the effect of age on substance P levels in man with most recent studies reporting receptor loss in central nervous system tissue with aging (39,41).

Strengths and limitations

Important study strengths include that we tested at multiple time-points during pain recovery; to the best of our knowledge, this has never been reported. Our testing protocol also limited the duration of the painful stimuli so that the biomarker cascade was not contaminated by repeated induction of early neuropeptide expression. Finally, our participants were carefully screened for health and instructed not to perform activities that are known to effect pain sensitivity and/or neuroendocrine or immune responses.

This study also has limitations. Plasma substance P and β -endorphin were not quantified at the site of stimulation. Also recognize that central versus circulating neuropeptides represent functionally different systems (2,3,14,59). For example, CNS β -endorphin release can also reduce pain via GABAergic inhibition. In addition, other neuropeptides that were not assessed may have supported or disconfirmed our conclusions. Current findings should not be construed as spinal and/or supraspinal response to pain, though it's probable that circulatory neuropeptides are at least partially influenced by central mechanisms. It is important to note that correlations such as those reported here can be overly influenced by several pairs of data. These findings need to be replicated in a larger sample.

Conclusions

Our study is one of the first to characterize time-course and magnitude of changes to these circulating neuropeptides following pain evocation in humans. There were mixed support for our age-related hypotheses with older adults demonstrating the anticipated stronger response to cold pain than for substance P, an excitatory neuropeptide. However, the directions of the findings for substance P in the HP model and for β -endorphin in the CPT were unexpectedly reversed. Future research will determine the extent to which neuropeptides change among individuals with clinical pain conditions, if maladaptive biomarker responses predispose individuals to prolonged acute pain, and/or the potential for pharmacologic agents to alter biomarker cascades based on age or pain stimulus.

References

1. Akil H, Shiomi H, Matthews J. Induction of the intermediate pituitary by stress: synthesis and release of a nonopioid form of beta-endorphin. *Science*. 1985; 227:424–6. [PubMed: 3155575]
2. Bach FW, Langemark M, Secher NH, Olesen J. Plasma and cerebrospinal fluid beta-endorphin in chronic tension-type headache. *Pain*. 1992; 51:163–8. [PubMed: 1484713]
3. Bäckryd E, Ghafouri B, Larsson B, Gerdle B. Do low levels of beta-endorphin in the cerebrospinal fluid indicate defective top-down inhibition in patients with chronic neuropathic pain? A cross-sectional, comparative study. *Pain Med*. 2014; 15:111–9. [PubMed: 24118997]
4. Bergman E, Johnson H, Zhang X, Hökfelt T, Ulfhake B. Neuropeptides and neurotrophin receptor mRNAs in primary sensory neurons of aged rats. *J Comp Neurol*. 1996; 375:303–19. [PubMed: 8915832]
5. Blandini F, Martignoni E, Melzi d'Eril GV, Biasio L, Sances G, Lucarelli C, Rizzo V, Costa A, Nappi G. Free plasma catecholamine levels in healthy subjects: a basal and dynamic study. The influence of age. *Scand J Clin Lab Invest*. 1992; 52:9–17. [PubMed: 1594891]
6. Casale G, Pecorini M, Cuzzoni G, de Nicola P. Beta-endorphin and cold pressor test in the aged. *Gerontology*. 1985; 31:101–5. [PubMed: 3158575]
7. Chiu IM, von Hehn CA, Woolf CJ. Neurogenic inflammation and the peripheral nervous system in host defense and immunopathology. *Nat Neurosci*. 2012; 15:1063–7. [PubMed: 22837035]
8. Cruz-Almeida Y, Aguirre M, Sorenson HL, Tighe P, Wallet SM, Riley JL 3rd. Age differences in cytokine expression under conditions of health using experimental pain models. *Exp Gerontol*. 2015; 72:150–6. [PubMed: 26456458]
9. Curatolo M, Petersen-Felix S, Arendt-Nielsen L. Sensory assessment of regional analgesia in humans – A review of methods and applications. *Anesthesiology*. 2000; 93:1517–30. [PubMed: 11149448]
10. Davis KD. Cold-induced pain and prickle in the glabrous and hairy skin. *Pain*. 1998; 75:47–57. [PubMed: 9539673]

11. De Souza EB, Van Loon GR. Rate-sensitive glucocorticoid feedback inhibition of adrenocorticotropin and beta-endorphin/beta-lipotropin secretion in rats. *Endocrinology*. 1989; 125:2927–34. [PubMed: 2555131]
12. Elahy M, Jackaman C, Mamo JC, Lam V, Dhaliwal SS, Giles C, Nelson D, Takechi R. Blood-brain barrier dysfunction developed during normal aging is associated with inflammation and loss of tight junctions but not with leukocyte recruitment. *Immun Ageing*. 2015; 12:2. [PubMed: 25784952]
13. Elliott AM, Smith BH, Penny KI, Smith WC, Chambers WA. The epidemiology of chronic pain in the community. *Lancet*. 1999; 354:1248–52. [PubMed: 10520633]
14. Facchinetti F, Petraglia F, Nappi G, Martignoni E, Antoni G, Parrini D, Genazzani AR. Different patterns of central and peripheral beta EP, beta LPH and ACTH throughout life. *Peptides*. 1983; 4:469–74. [PubMed: 6316295]
15. Fillingim RB, King CD, Ribeiro-Dasilva MC, Rahim-Williams B, Riley JL 3rd. Sex, gender, and pain: a review of recent clinical and experimental findings. *J Pain*. 2009; 10:447–85. [PubMed: 19411059]
16. Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci*. 2000; 908:244–54. [PubMed: 10911963]
17. Fruhstorfer H, Lindblom U. Vascular participation in deep cold pain. *Pain*. 1983; 17:235–41. [PubMed: 6657284]
18. Gibson SJ, Farrell M. A review of age differences in the neurophysiology of nociception and the perceptual experience of pain. *Clin J Pain*. 2004; 20:227–39. [PubMed: 15218407]
19. Gorlé N, Van Cauwenbergh C, Libert C, Vandenbroucke RE. The effect of aging on brain barriers and the consequences for Alzheimer's disease development. *Mamm Genome*. 2016; 27:407–20. [PubMed: 27143113]
20. Graven-Nielsen T, Arendt-Nielsen L. Peripheral and central sensitization in musculoskeletal pain disorders: an experimental approach. *Curr Rheumatol Rep*. 2002 Aug; 4(4):313–21. [PubMed: 12126583]
21. Greenhouse SW, Geisser S. On methods in the analysis of profile data. *Psychometrika*. 1959; 24:95–112.
22. Hargreaves KM, Dionne RA, Mueller GP. Plasma beta endorphin-like immunoreactivity, pain and anxiety following administration of placebo in oral surgery patients. *J Dent Res*. 1983; 62:1170–3. [PubMed: 6580319]
23. Hua S, Cabot PJ. Mechanisms of peripheral immune-cell-mediated analgesia in inflammation: clinical and therapeutic implications. *Trends Pharmacol Sci*. 2010; 31:427–33. [PubMed: 20591509]
24. Hughes AM, Rhodes J, Fisher G, Sellers M, Growcott JW. Assessment of the effect of dextromethorphan and ketamine on the acute nociceptive threshold and wind-up of the second pain response in healthy male volunteers. *Br J Clin Pharmacol*. 2002; 53:604–12. [PubMed: 12047485]
25. Johannes CB, Le TK, Zhou X, Johnston JA, Dworkin RH. The prevalence of chronic pain in United States adults: results of an Internet-based survey. *J Pain*. 2010; 11:1230–9. [PubMed: 20797916]
26. Julius D, Basbaum AI. Molecular mechanisms of nociception. *Nature*. 2001; 413:203–10. [PubMed: 11557989]
27. Kask A, Rageo L, Harro J. NPY Y-sub-1 receptors in the dorsal periaqueductal gray matter regulate anxiety in the social interaction test. *Neuroreport*. 1998; 9:2713–6. [PubMed: 9760107]
28. Kieffer BL, Evans CJ. Opioid receptors: from binding sites to visible molecules in vivo. *Neuropharmacology*. 2009; 56(Suppl 1):205–12. [PubMed: 18718480]
29. Klement W, Arndt JO. The role of nociceptors of cutaneous veins in the mediation of cold pain in man. *J Physiol*. 1992; 449:73–83. [PubMed: 1522527]
30. Kondo I, Marvizon JC, Song B, Salgado F, Codeluppi S, Hua XY, Yaksh TL. Inhibition by spinal mu- and delta-opioid agonists of afferent-evoked substance P release. *J Neurosci*. 2005; 25:3651–3660. [PubMed: 15814796]

31. LaMotte RH, Thalhammer JG. Response properties of high-threshold cutaneous cold receptors in the primate. *Brain Res.* 1982; 244:279–87. [PubMed: 7116176]
32. Latremoliere A, Woolf CJ. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. *J Pain.* 2009; 10:895–926. [PubMed: 19712899]
33. Lautenbacher S Experimental approaches in the study of pain in the elderly. *Pain Med.* 2012; 13(Suppl 2):S44–50. [PubMed: 22497747]
34. Littlejohn G. Neurogenic neuroinflammation in fibromyalgia and complex regional pain syndrome. *Nat Rev Rheumatol.* 2015; 11:639–48. [PubMed: 26241184]
35. Lovallo W. The cold pressor test and autonomic function: a review and integration. *Psychophysiology.* 1975; 12:268–82. [PubMed: 1153632]
36. Machelska H. Targeting of opioid-producing leukocytes for pain control. *Neuropeptides.* 2007; 41:355–63. [PubMed: 17640727]
37. Mauchly JW. Significance test for sphericity of n-variate normal population. *Annals of Mathematical Statistics.* 1940; 11:204–9.
38. McCormack K, Prather P, Chapleo C. Some new insights into the effects of opioids in phasic and tonic nociceptive tests. *Pain.* 1998; 78:79–98. [PubMed: 9839818]
39. Morley JE. Neuropeptides, behavior and aging. *J Am Geriatr Soc.* 1988; 34:52–62.
40. Muñoz M, Coveñas R. Involvement of substance P and the NK-1 receptor in human pathology. *Amino Acids.* 2014; 46:1727–50. [PubMed: 24705689]
41. Nyman MJ, Eskola O, Kajander J, Vahlberg T, Sanabria S, Burns D, Hargreaves R, Solin O, Hietala J. Gender and age affect NK1 receptors in the human brain – a positron emission tomography study with [18F]SPA-RQ. *Int J Neuropsychopharmacol.* 2007; 10:219–29. [PubMed: 16573846]
42. O'Connor TM, O'Connell J, O'Brien DI, Goode T, Bredin CP, Shanahan F. The role of substance P in inflammatory disease. *J Cell Physiol.* 2004; 201:167–80. [PubMed: 15334652]
43. Portenoy RK, Ugarte C, Fuller I, Haas G. Population-based survey of pain in the United States: differences among white, African American, and Hispanic subjects. *J Pain.* 2004; 5:317–28. [PubMed: 15336636]
44. Purves, D. Augustine, GJ. Fitzpatrick, D. Katz, LC. Lamantia, AS. McNamara, JO., Williams, M., editors. *Neuroscience.* 2nd. Sunderland (MA): Sinauer Associates; 2001.
45. Rohleder N, Wolf JM, Herpfer I, Fiebich BL, Kirschbaum C, Lieb K. No response of plasma substance P, but delayed increase of interleukin-1 receptor antagonist to acute psychosocial stress. *Life Sci.* 2006; 22(78):3082–9.
46. Rolandi E, Franceschini R, Marabini A, Messina V, Cataldi A, Salvemini M, Barreca T. Twenty-four-hour beta-endorphin secretory pattern in the elderly. *Acta Endocrinol (Copenh).* 1987; 115:441–6. [PubMed: 2820172]
47. Rosa C, Ghione S, Mezzasalma L, Pellegrini M, Fasolo B, Giaconi S, Gazzetti P, Ferdeghini M. Relationship between pain sensitivity, cardiovascular reactivity to cold pressor test, and indexes of activity of the adrenergic and opioid system. *Clin Exp Theory.* 1988; A10(Suppl 1):383–90.
48. Schedlowski M, Flüge T, Richter S, Tewes U, Schmidt RE, Wagner TO. Beta-endorphin, but not substance-P, is increased by acute stress in humans. *Psychoneuroendocrinology.* 1995; 20:103–10. [PubMed: 7530853]
49. Smith HS. Peripherally-acting opioids. *Pain Physician.* 2008; 11:S121–S132. [PubMed: 18443636]
50. Staud R. Evidence for shared pain mechanisms in osteoarthritis, low back pain, and fibromyalgia. *Curr Rheumatol Rep.* 2011; 13:513–20. [PubMed: 21833699]
51. Stein C, Zollner C. Opioids and sensory nerves. *Handb Exp Pharmacol.* 2009; 194:495–518.
52. Stein C. The Control of Pain in Peripheral Tissue by Opioids. *N Engl J Med.* 1995; 332:1685–1690. [PubMed: 7760870]
53. Stein C, Clark JD, Oh U, Vasko MR, Wilcox GL, Overland AC, Vanderah TW, Spencer RH. Peripheral mechanisms of pain and analgesia. *Brain Res Rev.* 2009; 60:90–113. [PubMed: 19150465]
54. Stein C, Schafer M, Machelska H. Attacking pain at its source: new perspectives on opioids. *Nat Med.* 2003; 9:1003–8. [PubMed: 12894165]

55. Strand, FL. *Neuropeptides Regulators of Physiological Processes*. 1st. Bradford: 1999.
56. Streff A, Kuehl LK, Michaux G, Anton F. Differential physiological effects during tonic painful hand immersion tests using hot and ice water. *Eur J Pain*. 2010; 14:266–72. [PubMed: 19540783]
57. Tassorelli C, Micieli G, Osipova V, Rossi R, Nappi G. Pupillary and cardiovascular responses to the cold-pressor test. *J Auton Nerv Syst*. 1995; 55:45–49. [PubMed: 8690850]
58. Varatharaj A, Galea I. The blood-brain barrier in systemic inflammation. *Brain Behav Immun*. 2016; 60:1–12. [PubMed: 26995317]
59. Veening JG, Gerrits PO, Barendregt HP. Volume transmission of beta-endorphin via the cerebrospinal fluid; a review. *Fluids Barriers CNS*. 2012; 9:16. [PubMed: 22883598]
60. Vierck CJ, Mauderli AP, Riley JL 3rd. Relationships between the intensity and duration of Peltier heat stimulation and pain magnitude. *Exp Brain Res*. 2013; 225:339–48. [PubMed: 23423165]
61. Yaksh TL. Substance P release from knee joint afferent terminals: modulation by opioids. *Brain Res*. 1988; 458:319–24. [PubMed: 2463049]
62. Yarnitsky D, Ochoa JL. Release of cold-induced burning pain by block of cold-specific afferent input. *Brain*. 1990; 113:893–902. [PubMed: 2397391]
63. Young E, Akil H. Changes in releasability of ACTH and beta-endorphin with chronic stress. *Neuropeptides*. 1985; 5:545–8. [PubMed: 2987742]
64. Young EA. Induction of the intermediate lobe pro-opiomelanocortin system with chronic swim stress and beta-adrenergic modulation of this induction. *Neuroendocrinology*. 1990; 52:405–14. [PubMed: 2175852]

Perspective

Older adults had higher baseline levels of substance P and β -endorphin suggesting increased peripheral activity compared to younger adults. Following 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.

Highlights

- Older adults had higher baseline levels of circulating substance P and β -endorphin compared to younger adults
- Older adults demonstrated a more intense initial response for both substance P and β -endorphin.
- The expected weaker response for β -endorphin among the older participants was not observed.
- Resolution generally occurred at similar time points for both younger and older adults.
- The response patterns differed between cold pressure pain and contact heat.

Figure 1. legend. Mean and standard error for baseline plasma neuropeptide concentrations collapsed across trials by age group. Group differences were significantly different for both substance P and β -endorphin at $p < .001$.

Figure 2. legend. Mean and standard error for change in plasma concentration from baseline following the cold pressor task by age group for substance P (Figure 2a) and β -endorphin (Figure 2b). * indicates group differences at $p < .05$.

Figure 3. legend. Mean and standard error for change in plasma concentration from baseline following contact heat pain by age group for substance P (Figure 3a) and β -endorphin (Figure 3b). * indicates group differences at $p < .05$.

Table 1

Mean SBP and DBP across the testing sessions for the total sample and by age group.

	Total sample	Younger	Older
	Mean (SD)	Mean (SD)	Mean (SD)
Cold pressor session			
SBP – baseline	113.8 (10.0)	111.5 (5.4)	115.8 (12.9)
SBP – stimuli administration	121.4 (14.1)	121.4 (14.6)	121.6 (14.6)
SBP – discharge	119.5 (17.1)	109.8 (7.7)	128.1 (18.8)
DBP – baseline	68.8 (9.2)	68.0 (8.9)	69.7 (10.0)
DBP – stimuli administration	73.3 (12.7)	74.1 (13.6)	72.6 (12.6)
DBP – discharge	72.8 (11.1)	67.1 (6.9)	77.9 (12.0)
Heat pain session			
SBP – baseline	117.9 (14.5)	111.4 (12.0)	123.8 (14.5)
SBP – stimuli administration	123.4 (14.5)	116.5 (11.7)	129.7 (14.4)
SBP – discharge	119.5 (14.5)	114.9 (10.6)	123.1 (16.6)
DBP – baseline	67.2 (9.5)	62.0 (3.5)	71.8 (10.8)
DBP – stimuli administration	70.2 (13.8)	61.3 (9.1)	78.2 (12.4)
DBP – discharge	69.4 (11.2)	64.8 (8.8)	73.4 (12.0)

Table 2

Mean and standard deviations for plasma concentrations at baseline and change from baseline for Substance P for each session by time-point.

	Baseline	3-min	15-min	30-min	45-min	60-min	90-min
	Mean (SD)	Mean* (SD)	Mean* (SD)	Mean* (SD)	Mean* (SD)	Mean* (SD)	Mean* (SD)
Cold pressor session							
Younger	0.9 (0.1)	6.3 (0.7)	4.8 (0.5)	4.8 (0.5)	5.1 (0.5)	5.6 (0.6)	-0.7 (0.1)
Older	3.5 (0.7)	22.7 (4.5)	8.9 (2.9)	2.8 (0.6)	2.6 (0.5)	5.4 (1.1)	-2.9 (0.6)
p. value	< .001	< .001	.002	< .001	< .001	> .05	< .001
Contact heat session							
Younger	0.6 (0.1)	25.5 (1.7)	24.0 (1.8)	21.7 (1.8)	0.6 (0.6)	1.8 (0.6)	1.1 (0.8)
Older	4.6 (0.9)	16.4 (2.1)	18.9 (2.3)	27.7 (3.8)	-2.7 (2.0)	-1.5 (2.5)	-1.7 (0.9)
p. value	< .001	< .001	< .001	.001	.001	.003	> .05

* Change from baseline in pg/ml.

Table 3

Mean and standard deviations for plasma concentrations at baseline and change from baseline for B- endorphin for each session by time-point.

	Baseline	3-min	15-min	30-min	45-min	60-min	90-min
	Mean (SD)	Mean* (SD)	Mean* (SD)	Mean* (SD)	Mean* (SD)	Mean* (SD)	Mean* (SD)
Cold pressor session							
Younger	1.4 (0.2)	40.8 (4.2)	52.6 (5.4)	5.6 (0.6)	8.9 (0.9)	3.7 (0.4)	0.5 (0.1)
Older	6.7 (1.3)	43.3 (8.5)	87.3 (17.0)	6.4 (1.3)	6.6 (1.3)	3.5 (0.7)	-1.9 (0.4)
p. value	< .001	> .05	< .001	> .05	.001	> .05	< .001
Contact heat session							
Younger	0.3 (0.1)	29.7 (2.0)	30.6 (2.0)	47.6 (7.2)	1.0 (0.3)	1.5 (.24)	0.6 (0.7)
Older	6.8 (0.9)	13.5 (1.6)	27.5 (3.3)	49.8 (7.1)	-5.0 (2.0)	-4.5 (1.4)	-5.6 (1.2)
p. value	< .001	< .001	.032	> .05	< .001	< .001	< .001

* Change from baseline in pg/ml.

Table 4

Correlations between baseline levels and changes in plasma concentrations for the HP model

	Younger n=8		Older n=9	
	Baseline substance P	Baseline β -endorphin	Baseline substance P	Baseline β -endorphin
β -endorphin 3-min	.92 **	.95 **	.56	.81 **
β -endorphin 15-min	.99 **	.98 **	.71 *	.90 **
β -endorphin 30-min	.27	.36	.96 **	.99 **
β -endorphin 45-min	.42	.32	-.92 **	-.74 *
β -endorphin 60-min	.23	.32	-.96 **	-.80 **
substance P 3-min	.99 **	.98 **	.29	.59
substance P 15-min	.94 **	.91 **	.65 *	.87 **
substance P 30-min	.89 **	.83 **	.94 **	.99 **
substance P 45-min	.25	.15	-.86 **	-.64 *
substance P 60-min	.03	.13	-.81 **	-.57

**
Key, < .01,*
< .05