Aging, opioid-receptor agonists and antagonists, and the vestibulosympathetic reflex in humans

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Ray, Chester A., and Kevin D. Monahan. Aging, opioid-receptor agonists and antagonists, and the vestibulosympathetic reflex in humans. J Appl Physiol 96: 1761–1766, 2004.—Animal studies indicate that the vestibular system reflexively alters sympathetic nerve activity (15, 39) and regional blood flow (18) and contributes critically to orthostatic blood pressure regulation (8, 16). Consistent with these experimental findings in animals, head-down rotation (HDR) performed in young adults, which engages the otolith organs of the vestibular system, increases muscle sympathetic nerve activity (MSNA) and produces limb vasoconstriction (14, 20, 25, 34). These findings in young humans are consistent with the concept that vestibular activation contributes to orthostatic blood pressure regulation. Importantly, HDR performed in older subjects elicits an attenuated reflex increase in MSNA and systemic hypotension (21, 27). These latter findings suggest that impairment in the vestibulosympathetic reflex may compromise orthostatic blood pressure regulation in older adults.

Consistent with these suggestions, the incidence of orthostatic hypotension increases with advancing age in humans (31). Thus impairment of the vestibulosympathetic reflex (21, 27) may contribute mechanistically to this increased prevalence of orthostatic hypotension with age and emphasizes the clinical importance of determining factors underlying its impairment. One such potential mechanism involves opioids. Specifically, opioid agonist administration elicits physiological responses in the vestibular nuclei of the brain stem (2, 5, 11), which is critical in mediating the vestibulosympathetic reflex in the cat (40). Furthermore, opioid agonists inhibit neuronal firing rates in the vestibular nuclei in an age-dependent manner in rats with the greatest effect being demonstrated in more mature rats (37). Presently it is unknown whether opioid agonist or antagonist can modify the vestibulosympathetic reflex in humans. Moreover, if opioids do tonically inhibit the vestibulosympathetic reflex, it is not known whether greater inhibition occurs in older adults and thus contributes to the age-associated impairment in the vestibulosympathetic reflex.

Accordingly, we tested the hypotheses that 1) opioid-receptor agonists and antagonists modulate the vestibulosympathetic reflex in humans and 2) endogenous opioid inhibition of the vestibulosympathetic reflex is greater in older adults and thus contributes mechanistically to the age-associated impairment in the vestibulosympathetic reflex (21, 27). To test these hypotheses, we compared MSNA responses to HDR before and after administration of an opioid-receptor antagonist (naloxone) and agonist (codeine) in young and older subjects.

METHODS

Subjects

Thirty-one (16 young and 15 older) healthy normotensive volunteers were studied prospectively. On the basis of age, subjects were classified as either “young” (20–35 yr) or “older” (55–70 yr). Subjects were randomly assigned to receive either naloxone (9 young and 8 older subjects) or codeine (7 young and 7 older subjects). Written informed consent was obtained from all subjects after verbal explanation of the experimental protocols. The experimental protocols were approved by the Institutional Review Board at the Pennsylvania State University College of Medicine.

Experimental Design

Study 1 (naloxone trial). The purpose of this study was to determine whether endogenous opioids tonically inhibit the vestibulosym...
pathetic reflex in young and older subjects. To address this aim, subjects (9 young and 8 older) performed HDR in the prone position as described previously (34). HDR was used to selectively activate the otolith organs of the vestibular system. Our laboratory has previously demonstrated that responses to HDR appear to be independent of the influences of neck muscle afferents, baroreflexes, central command, visual input, and increased intracranial pressure (14, 25, 26, 34). This experimental protocol began at least 30 min after insertion of an intravenous cannula into an antecubital vein. Briefly, subjects were positioned prone on an examination table with their head extending over the end of the table, such that the head could be rotated downward without interference from the end of the table. During baseline, the subject’s head was supported in the chin-up neck extended position. After this 3-min baseline period, the chin support was removed and the subject’s head was passively lowered to the point of maximal rotation. After a 1-min period of HDR, the subject’s head was passively returned to the baseline position for a 3-min period of recovery. After this first HDR protocol, the opioid-receptor antagonist naloxone hydrochloride (Elkins-Sinn, Cherry Hill, NJ) (16 mg) was administered intravenously as a bolus over ~60 s. This dose of naloxone was chosen because it has previously been shown to exert biological actions while minimizing the likelihood of side effects associated with larger doses (29). Because naloxone was administered intravenously and because it exerts its effect very rapidly, the second bout of HDR was performed shortly (5 min) after naloxone administration. The second HDR trial was repeated in an identical fashion to trial 1.

Study 2 (codeine trial). The purpose of this study was to determine whether administration of an opioid-receptor agonist (codeine) inhibits the reflex increase in MSNA during otolith organ engagement in young and older subjects. To address this aim, subjects (7 young and 7 older) performed HDR before and after administration of codeine. As in study 1, HDR was performed in prone subjects by rotating their head downward from the end of an examination table. Each study began with the subject’s head in the baseline chin-up supported position. After a 3-min baseline period, HDR was performed for 1-min. After this HDR period the head was returned to the baseline chin-up position for a 3-min recovery period. After this first trial of HDR, an opioid-receptor agonist (60 mg codeine, Roxane, Columbus, OH) was ingested orally at a dose previously shown to exert biological actions without apparent side effects (1, 23). Sixty minutes after codeine ingestion, subjects repeated the HDR trial as prior to the codeine ingestion.

MSNA, mean arterial blood pressure, and heart rate were measured continuously during both experiments and all trials.

Measurements

Multifiber recordings of MSNA were obtained by inserting a tungsten microelectrode in the peroneal nerve of the leg. A reference electrode was inserted subcutaneously in close proximity to the recording electrode. The recording electrode was adjusted until a site with clear spontaneously occurring sympathetic bursts was established. Standard criteria for acceptable recordings of MSNA were applied (38). Raw nerve recordings were amplified (20,000–70,000 times), filtered (700–2,000 Hz), full-wave rectified, and integrated (0.1-s time constant) to obtain mean voltage neurograms.

Continuous measurements of arterial blood pressure and heart rate were made by using a Finapres photoplethysmograph (Ohmeda, Louisville, CO). Mean voltage neurograms, heart rate, and arterial blood pressure tracings were collected (MacLab 8e, ADInstruments) on computer for visual inspection and later analyses.

Data Analysis

Sympathetic bursts were visually identified from the integrated mean voltage neurograms. MSNA is reported as both burst frequency (bursts/min) and total activity (sum of the amplitude of individual bursts) as measured by a computer program (Peaks, ADInstruments, Milford, MA). The technician performing all analyses was blinded as to which group or condition the subject belonged.

A one-between (drug) and one-within (intervention) repeated-measures analysis of variance was used to determine the effect of HDR on both MSNA and cardiovascular measures. Planned comparisons were used to identify specific contrasts that differed significantly. Statistical significance was set at $P < 0.05$. Values are presented as means ± SE throughout.

RESULTS

Study 1

Older subjects (62 ± 2 yr old; 5 men and 3 women) demonstrated elevated levels of MSNA at baseline compared with young subjects (24 ± 1 yr old; 6 men and 3 women) in study 1 (14 ± 2 vs. 36 ± 5 bursts/min for young and older subjects, respectively; $P < 0.05$). Before intravenous administration of naloxone, HDR significantly increased MSNA in young ($Δ7 ± 2 bursts/min and $Δ81 ± 23%$ for burst frequency and total MSNA, respectively) but not older subjects ($Δ2 ± 2 bursts/min and $Δ8 ± 7%$) (Fig. 1). HDR performed after naloxone administration increased MSNA to a similar extent as during the control HDR trial in both young ($Δ4 ± 2 bursts/min and $Δ60 ± 24%$) and older subjects ($Δ1 ± 2 bursts/min and $Δ8 ± 9%$) (Fig. 1). Naloxone did not alter baseline levels of MSNA ($14 ± 2 vs. 14 ± 2 and 36 ± 5 vs. 38 ± 4 bursts/min in young and older subjects before and after naloxone, respectively), Finapres-derived mean arterial blood pressure (79 ± 3 vs. 80 ± 3 and 103 ± 4 vs. 102 ± 5 mmHg), or heart rate (61 ± 3 vs. 60 ± 3 and 65 ± 3 vs. 64 ± 3 beats/min). Moreover, HDR performed after naloxone administration did not alter the Finapres-derived mean arterial blood pressure and heart rate responses (Fig. 1). All variables returned to baseline levels during recovery.

Study 2

As in study 1 baseline levels of MSNA were significantly elevated in older (65 ± 1 yr old; 4 men and 3 women) compared with younger (25 ± 1 yr old; 6 men and 1 woman) subjects (16 ± 3 vs. 29 ± 6 bursts/min for young and older subjects, respectively). Before codeine administration HDR increased MSNA in young ($Δ8 ± 1$ and $Δ53 ± 4%$ for burst frequency and total MSNA, respectively; $P < 0.05$ from baseline) and older ($Δ6 ± 4 and $Δ38 ± 21%$; $P < 0.05$ from baseline) subjects (Fig. 2). HDR performed 1 h after codeine ingestion did not significantly alter MSNA responses to HDR in young ($Δ7 ± 2 and $Δ64 ± 16%$) or older subjects ($Δ3 ± 3$ and $Δ33 ± 20%$) (Fig. 2). In young subjects, codeine did not alter baseline levels of MSNA (17 ± 3 vs. 19 ± 5 bursts/min before and after codeine, respectively), Finapres-derived mean arterial blood pressure (75 ± 2 vs. 76 ± 2 mmHg), or heart rate (59 ± 4 vs. 57 ± 4 beats/min). In older subjects, MSNA (29 ± 6 vs. 34 ± 4 bursts/min before and after codeine, respectively) and Finapres-derived mean arterial blood pressure (97 ± 4 vs. 114 ± 11 mmHg) were significantly elevated after codeine ingestion. Heart rate was not altered by codeine (71 ± 3 vs. 70 ± 2 beats/min). Additionally, the Finapres-derived mean arterial blood pressure and heart rate responses to HDR were not altered by codeine (Fig. 2). All variables returned to baseline levels during recovery.
No discernible physical or subjective side effects were noted by any of the young and older subjects with the administration of either naloxone or codeine. Additionally, we detected no apparent gender influence on any of the noted responses. The angle of head rotation did not differ between young (86 ± 4°) and older (78 ± 6°) subjects as previously demonstrated (27).

**DISCUSSION**

The primary new finding from the present study is that opioids do not appear to modulate the vestibulosympathetic reflex in humans. The main experimental support for this statement comes from the demonstration that neither the opioid-receptor antagonist naloxone nor the opioid-receptor agonist codeine altered the reflex increase in MSNA during otolith organ engagement in either young or older humans. Thus, contrary to our hypothesis, the present data indicate that opioid inhibition of the vestibulosympathetic reflex does not contribute to the age-associated impairment of this reflex.

Consistent with our previous findings, the present data confirm that aging is associated with both an attenuated increase in MSNA and hypotension during otolith organ engagement (21, 27). It is possible that both the attenuated increase in MSNA and the additional challenge of hypotension that occurs during otolith organ engagement in older subjects contribute to the increased prevalence of orthostatic hypotension with age in humans. Thus identification of the mechanism(s) underlying the age-associated impairment of the vestibulosympathetic reflex is of clinical importance. In this context, aging is associated with anatomic and functional alterations within the vestibular system that may contribute to a diminished vestibu-
osympathetic reflex with age. These changes include, but are not limited to, a reduction in the number of afferent projections from and reduced number of hair cells within the vestibular apparatus (10, 19, 30, 36). Alterations in the vestibular system of these types with age likely alter vestibular function. Consistent with these experimental findings, both the vestibuloocular and vestibulospinal reflexes are impaired with age in humans (12, 22, 35). Thus it is likely that anatomic and functional changes within the vestibular system contribute to the attenuated increases in MSNA during otolithic engagement with age in humans. However, it is possible that other mechanisms contribute to the age-related impairment in the vestibulosympathetic reflex in humans.

Opioid-receptor binding sites have been identified in the vestibular nuclei of the brain stem (2, 5, 11). Importantly, the vestibular nuclei has been demonstrated to be critical in mediating the vestibulosympathetic reflex in the cat (40). Furthermore, electrophysiological studies indicate that opioid agonists inhibit the firing rate of vestibular neurons (17). Additionally, inhibition of vestibular neuron firing rate by opioids is greater in more mature rats (37). On the basis of these experimental data, it appeared possible that endogenous opioids tonically inhibit the vestibulosympathetic reflex in humans with greater inhibition occurring in older subjects. This potential for greater inhibition of the vestibulosympathetic reflex, by endogenous opioids, with age may contribute mechanistically to the age-
associated impairment in the vestibulosympathetic reflex. Importantly, naltrexone has previously been demonstrated to alter the physiological responses to vestibular activation in animals (9). However, the present experimental findings do not support an obligatory role for opioids in modulating the vestibulosympathetic reflex in young or, more importantly, older adults. As such, it is unlikely that endogenous opioids contribute to the age-related impairment of the vestibulosympathetic reflex in humans.

It is likely the differences between the animal studies and the present study with regard to the effect of opioid on the vestibular system are related to possible species differences and the nature of the experimental designs (i.e., cellular vs. integrative). However, several limitations in the present study deserve mention. First, it is possible that the lack of effect of naltrexone on the vestibulosympathetic reflex in older subjects is mediated by a reduction in basal endogenous opioid levels with age. Presently, the effect of age on endogenous opioid level is equivocal (3, 4, 7, 32). Thus it is conceivable that an age-related reduction in basal endogenous opioid levels contributed to the lack of observable effect of naltrexone on the vestibulosympathetic reflex in older subjects. However, this may be less likely because administration of the opioid-receptor agonist codeine did not impair the vestibulosympathetic reflex in either young or older subjects. Studies in the rat indicate that the affinity of the opioid receptors does not appear to be altered by age (13). Thus it is unlikely that the lack of effect of codeine on the vestibulosympathetic reflex in older subjects was mediated by altered drug kinetics or receptor binding of codeine secondary to the effects of age. Collectively, these findings and the findings from the naltrexone trial indicate that opioids likely do not modulate the vestibulosympathetic reflex in humans.

Second, it is possible that higher doses of opioid receptor agonist may impair the vestibulosympathetic reflex. The present data cannot exclude this possibility. However, importantly, the failure of codeine to alter MSNA responses to HDR in the older subjects suggest that reduced endogenous opioid levels likely do not explain the lack of effect of naltrexone in the older subjects.

It has been previously shown that intravenous infusion of naltrexone of 0.15 mg/kg alters cardiopulmonary baroreflex control of MSNA in humans (33). This finding indicates the dose of naltrexone used in the present study (≥0.20 mg/kg) had sufficient capacity to elicit physiological effects. Therefore, the dosage of naltrexone is unlikely to explain its lack of effect on the vestibulosympathetic reflex in older subjects observed in the present study. Additionally, it is possible that vestibular effects of naltrexone and codeine were obscured by concomitant action of these drugs on other aspects of autonomic nervous system reflex function, such as ganglionic transmission. However, this appears unlikely because our laboratory has previously demonstrated that neither naltrexone nor codeine alters sympathetic responses to physiological stressors such as exercise (6, 28).

It is interesting to note that older but not younger subjects demonstrated an increase in MSNA after codeine ingestion. It is unlikely that the heightened level of sympathoexcitation after codeine ingestion influenced responses to otolith organ activation. Importantly, our laboratory has previously demonstrated that elevating the baseline levels of MSNA or arterial blood pressure before HDR does not alter responses to HDR (24, 27). Presently, it is unclear why MSNA was increased after codeine ingestion in older subjects. Older subjects did express that they had some discomfort while lying in the prone position for the 60-min period during the codeine trial. It is unlikely that discomfort alone could have mediated the increases in MSNA and arterial blood pressure, entirely. Animal studies suggest that age-related changes in receptor-mediated responsiveness to codeine is unlikely to explain these results because opioid-receptor affinity is not influenced by age as determined in the rat (13). The sympathoexcitation observed with codeine in the older subjects might warrant caution for its use as an analgesic in the elderly. Studies that specifically address this issue are needed.

In summary, the present findings do not provide experimental support for the concept that opioids modulate the vestibulosympathetic reflex in humans. These conclusions are supported by the lack of effect of the opioid-receptor antagonist naltrexone and the opioid-receptor agonist codeine on the reflex increases in MSNA during otolith organ engagement in humans. Equally as important, the present findings demonstrate that, despite indirect evidence in the animal literature, endogenous opioids likely do not contribute to the age-related impairment in the vestibulosympathetic reflex. These findings suggest that anatomic and functional changes occurring in the vestibular system with age are likely to be primary contributors of the age-related impairment in the vestibulosympathetic reflex in humans.

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