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Diving and exercise: The interaction of trigeminal receptors and muscle metaboreceptors on muscle sympathetic nerve activity in humans


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FACIAL IMMERSION IN COLD WATER evokes a parasympathetically mediated decrease in heart rate (HR) and a sympathetically mediated increase in blood pressure and peripheral vasconstriction (11, 16, 22). This “diving response” plays an important role in conserving oxygen, and, while it is most powerful in diving mammals and birds (3, 12, 49), a similar, albeit less marked, phenomena also occurs in humans (15, 20, 26). The stimulation of afferent fibers of the trigeminal nerve underpins the neural cardiovascular responses to cold-water face immersion (16) but may be modified by other sensory stimuli, such as those associated with apneic submersion (e.g., pulmonary afferents, chemoreflex) (22). Exercise can also modify the cardiac parasympathetic response to trigeminal nerve stimulation (2), but how sympathetic responses to trigeminal nerve stimulation are changed by exercise is unknown. This is important because the powerful and concurrent activation of the sympathetic and parasympathetic branches of the autonomic nervous system (“autonomic conflict”) may increase the risk of lethal arrhythmia (42), particularly during athletic events such as open water swimming (12, 44).

In contrast to the diving response, exercise is characterized by an intensity-dependent reduction in cardiac parasympathetic activity and increase in sympathetic activity to the heart and peripheral vasculature. Such autonomic alterations augment cardiac chronotropic and inotropic state, thus increasing cardiac output and facilitating its redistribution to the active muscles. Such autonomic responses are orchestrated by several neural mechanisms, including central command, comprising feed-forward signals arising from the brain, and the exercise pressor reflex, comprised of sensory feedback from group III and IV skeletal muscle afferents responsive to metabolic (metaboreflex) and mechanical (chemoreflex) stimulation (13, 19, 23, 31). Al-Ani et al. (2) observed that the magnitude of the reduction in R-R interval (increase in HR) at the onset of high-intensity isometric handgrip is attenuated by concurrent trigeminal nerve stimulation (facial cooling) and further suggested that the conflicting effects of trigeminal nerve stimulation and exercise on cardiac parasympathetic activity algebraically summate. Central command plays an important role in the increases in HR at the onset of exercise (32), and the parasympathetically mediated bradycardic response to facial cooling is reportedly attenuated by central command at the onset of exercise, rather than by skeletal muscle afferents (43).
How the muscle sympathetic nerve activity (MSNA) responses to trigeminal nerve stimulation are modified by exercise has not been investigated. The main stimulus for increase in MSNA during exercise is the muscle metaboreflex (30, 33, 47), but whether excitatory inputs from the trigeminal nerve and metabolically sensitive skeletal muscle afferents summate algebraically in determining a MSNA response, or exhibit synaptic occlusion or sensitization, is unknown. The question is important because the extent of convergence of the two afferent inputs will affect the degree of increase in peripheral resistance, hence cardiac work, and the redistribution of cardiac output during swimming activity. Furthermore, it widens the knowledge of cardiovascular reflex interactions that may impact optimal physical performance and also survival in water.

The study examined the interaction between the trigeminal nerve reflex using the “cold face test” and muscle metaboreceptor reflex, which was isolated by posthandgrip exercise ischemia (PEI). Blood pressure, MSNA, and femoral artery vascular conductance were measured during the following three experimental conditions: 1) facial cooling (0°C; trigeminal nerve stimulation), 2) isometric handgrip followed by PEI, and 3) trigeminal nerve stimulation with isometric handgrip and PEI. HR, HR variability, and cardiac baroreflex sensitivity analyses were employed to assess cardiac autonomic function. We sought to determine whether excitatory inputs from the trigeminal nerve and the muscle metaboreflex summate algebraically in determining a MSNA response.

MATERIALS AND METHODS

Subjects

All experimental protocols conformed to the Declaration of Helsinki and were approved by the ethical committee of the Faculty of Medicine Ethical Committee for Research at Fluminense Federal University (CAAE: 09282812.30000.5243). A detailed verbal and written explanation of the study was provided to each subject following which written informed consent for participation was provided. Eight male subjects participated in the present study with an age, weight, and height of 30 ± 2 yr, 79 ± 3 kg, and 179 ± 2 cm (mean ± SE), respectively. All subjects were in good health and were not taking any prescription or over-the-counter medications. Subjects abstained from consuming caffeine and alcohol and refrained from exercise for 24 h before arrival at the laboratory. All study measurements were made in a temperature-controlled room (20–22°C).

Measurements

Blood pressure was continuously and noninvasively monitored using finger photoplethysmography (Finometer Pro; Finapres Medical Systems, Arnhem, The Netherlands) on the left hand. Resting values were verified by brachial artery blood pressure measurements obtained from the right arm using an automated sphygmomanometer. HR was assessed using a lead II electrocardiogram (BioAmp, MLA2540; ADInstruments, Bella Vista, NSW, Australia). Respiratory-related changes in thoracic circumference were monitored using a piezoelectric transducer (model 1132 Pneumotrace II; UFI, Morro Bay, CA). Postganglionic multisite MSNA was obtained using a unipolar tungsten microelectrode inserted in the peroneal nerve of the right leg at the fibular head, and a reference electrode was placed subcutaneously 2–3 cm distal. The recording electrode was adjusted until a site was found where nerve unit activity displayed a plesysynchronous pattern of spontaneous bursts, had a signal-to-noise ratio of 3:1, was increased during an end-expiratory breath-hold or Valsalva maneuver, and was unresponsive to an unexpected loud noise or skin stroking (45). The raw signal was amplified (×100,000), filtered (bandwidth 700–2,000 Hz), rectified, and integrated (time constant 0.1 s) to obtain a mean voltage neurogram (Iowa Bioengineering, Iowa City, IA). Sympathetic bursts were identified using a fully automated program (24). The mean voltage neurogram was normalized by calibrating the height of the largest set of bursts during baseline to a value of 1,000 arbitrary integration units (AU). MSNA was quantified as burst incidence (bursts/100 heart beats), burst frequency (bursts/min), and MSNA total activity (AU).

Femoral blood flow velocity (FBV) from the right leg was obtained by Doppler ultrasound (Logiq P5; GE Medical Systems, Milwaukee, WI), using a 10-MHz multifrequency linear-array transducer. FBV was measured over the femoral artery with the linear array Doppler probe in Duplex mode, with a constant insonation angle of 60° relative to the skin, and stored on the ultrasound device for offline analysis. The Doppler ultrasound video signal was captured at a frequency of 30 Hz using a video capture board with an audio USB 2.0 (EasyCap DC60; Leadership) connected to a computer. The video files were compatible with commercial automated edge-detection and wall-tracking software (Vascular Research Tools 5; Medical Imaging Applications), which was used for offline analysis. In the initial phase of software analysis, regions of interest were identified at the optimal portion of the femoral artery image and blood velocity spectra. Brachial artery diameter and blood velocity were then continuously assessed (i.e., an R wave gaiting function was not applied) (9). Femoral blood flow (FBF; in ml/min) was calculated as FBF × π × (diameter/2)² × 60, and femoral vascular conductance (FVC; in ml·min⁻¹·mmHg⁻¹) was calculated as FBF/mean arterial pressure (MAP).

Protocol

Subjects rested in a supine position on a medical examination bed with a handgrip dynamometer held in the right hand (MLT004/ST; ADInstruments). A cuff was placed around the upper arm, to be rapidly inflated to a suprasystolic pressure (220 mmHg) thus occluding the forearm circulation as required. Subjects attempted three to five maximal isometric handgrip efforts each separated by at least 1 min (the maximal voluntary contraction (MVC) was taken as the highest force produced. Subjects were then instrumented and rested quietly for ~10 min during which respiratory rate and depth were observed. A metronome was set to their natural respiratory frequency, and the subjects then practiced breathing while guided by this, with adjustments made as appropriate. Subjects were also provided with a display of their respiratory movements and requested to maintain a consistent tidal volume.

On a single experimental session, subjects undertook the following three protocols. The order of the trials was counterbalanced and separated by an ~20-min rest period.

Protocol 1: Trigeminal nerve stimulation. Following a 3-min rest period, an ice pack (0°C) shaped to cover the areas innervated by the ophthalmic (forehead) and maxillary divisions (cheeks) of the trigeminal nerve was used to simulate the diving response for 3 min (2). The ice pack adapted to the contours of the face and thus provided a good contact with the skin. This procedure was followed by a 3-min recovery period. Respiratory pattern was guided throughout as described above. Mean cardiovascular and MSNA data were obtained at rest (3 min). Trigeminal stimulation data were obtained for the first (onset) and last (end) 15 s of the 3-min stimulation period. This strategy permitted the subsequent evaluation of trigeminal stimulation during isometric handgrip and PEI as described below.

Protocol 2: Isometric handgrip and selective activation of muscle metaboreflex (PEI). Subjects rested quietly for 3 min following which they performed 3 min of isometric handgrip at 25% MVC. The force evoked was displayed on a screen to provide visual feedback. Fifteen seconds before the termination of the contraction, the upper arm cuff
was rapidly inflated to 220 mmHg. This remained inflated for a further 3 min (PEI) to isolate the muscle metaboreflex after the isometric handgrip ceased. Following cuff deflation, a 3-min recovery period was conducted. A rating of perceived exertion (RPE) was obtained using the 0–10 Borg scale during isometric handgrip (10). Respiratory frequency (metronome) and depth (visual display) were guided at rest, PEI, and recovery but not during isometric handgrip. Mean cardiovascular data were obtained at rest (3 min), isometric handgrip (last 15 s), PEI (last 15 s), and recovery (3 min).

Protocol 3: Simultaneous trigeminal nerve stimulation during isometric handgrip and selective activation of muscle metaboreflex (PEI). Following a 3-min rest period, subjects performed 3 min of isometric handgrip at 25% MVC. Fifteen seconds before the termination of the contraction, an ice pack (0°C) was placed on the face to evoke trigeminal nerve stimulation, and the upper arm cuff was rapidly inflated to 220 mmHg. Both the ice pack (trigeminal nerve stimulation) and the upper arm cuff inflation (isolated muscle metaboreflex activation with PEI) were continued for a further 3 min after cessation of voluntary contraction, following which both stimuli were removed and a 3-min recovery period was conducted. RPE was obtained once again during isometric handgrip using the 0–10 Borg scale (10). Respiratory frequency (metronome) and depth (visual display) were guided at rest, during PEI with trigeminal nerve stimulation and recovery, but not during isometric handgrip. Mean cardiovascular and MSNA data were obtained at rest (3 min), trigeminal nerve stimulation with handgrip (last 15 s of handgrip), trigeminal nerve stimulation with PEI (last 15 s), and recovery (3 min).

Data Analysis

The raw signals were sampled at 1 kHz and stored for offline analysis (LabChart 7 Pro and Powerlab; ADInstruments). HR, R-R interval, and systolic and diastolic blood pressure were obtained on a beat-to-beat basis. MAP was obtained by integration of the arterial blood pressure waveform over a cardiac cycle. An estimate of cardiac parasympathetic activity was derived from the square root of the mean of successive differences in R-R interval (RMSSD) (43a), and an assessment of cardiac baroreflex sensitivity was made using the sequence technique (CardioSeries version 2.2, São Paulo, SP, Brazil) (35). As described previously, the sequence technique involved the analysis of beat-to-beat time series of systolic blood pressure and R-R interval using a customized algorithm to identify sequences of three or more beats where these variables changed in the same direction (35). A linear regression was then applied to each systolic blood pressure-R-R interval sequence, and an index of cardiac baroreflex sensitivity provided from the mean slope of those sequences with an $r^2 > 0.85$. For statistical analyses, average values for cardiac baroreflex sensitivity were obtained over 3 min of rest, trigeminal nerve stimulation, PEI, and trigeminal nerve stimulation with PEI.

Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences software, version 19.0 for Windows (Chicago, IL). Normal distribution was evaluated using Shapiro-Wilk tests. Comparisons of normally distributed physiological variables within each protocol were made using repeated-measures ANOVA, and non-normally distributed data were evaluated using Friedman ANOVA. Post hoc comparisons were performed using either paired t-test or Wilcoxon signed test with Bonferroni adjustment. To examine the interactive effects of trigeminal nerve stimulation and isometric handgrip, the percentage change in MAP, HR, FVC, RMSSD and MSNA during trigeminal nerve stimulation (first 15 s), isometric handgrip (last 15 s), and trigeminal nerve stimulation with isometric handgrip (first 15 s of trigeminal nerve stimulation combined with the last 15 s of isometric handgrip) were compared using one-way ANOVA. One-way ANOVA was also used to compare the percentage change in MAP, HR, FVC, RMSSD, and MSNA during trigeminal nerve stimulation (last 15 s), PEI (last 15 s), and trigeminal nerve stimulation with PEI (last 15 s) to establish the interactive effects of trigeminal nerve stimulation and muscle metaboreflex activation. The percentage change for each variable was calculated as its absolute change from rest during the experimental intervention (e.g., PEI), divided by the rest value, and multiplied by 100. Data are presented as means $\pm$ SE. A $P$ value of $<0.05$ was considered statistically significant.

RESULTS

The neural and cardiovascular responses to trigeminal nerve stimulation (protocol 1) are shown in Table 1, and an original record from one subject is shown in Fig. 1. Trigeminal nerve stimulation resulted in elevation of MAP ($\Delta$14 $\pm$ 3 mmHg) and MSNA (total activity, $\Delta$3,941 $\pm$ 1,212 AU; $P < 0.05$), whereas FBF remained unchanged from rest and FVC decreased ($\Delta$−1.23 $\pm$ 0.42 ml·min$^{-1}$·mmHg$^{-1}$; $P < 0.05$). During trigeminal nerve stimulation, RMSSD was significantly increased ($\Delta$47 $\pm$ 34 ms), R-R interval tended to increase ($P = 0.09$), and HR tended to decrease ($P = 0.18$). Cardiac baroreflex sensitivity numerically, but nonsignificantly, increased from 22 $\pm$ 3 at rest before trigeminal nerve stimulation to 27 $\pm$ 7 ms/mmHg during trigeminal nerve stimulation before falling to 16 $\pm$ 1 ms/mmHg in the recovery period.

### Table 1. Neural cardiovascular values during trigeminal nerve stimulation with facial cooling

| & Rest & Onset & End & Recovery & $P$ Value |
|---|---|---|---|---|---|
| Systolic BP, mmHg | 122 $\pm$ 4 | 128 $\pm$ 4 | 143 $\pm$ 8$^\dagger$ | 123 $\pm$ 5$^\dagger$ | $<0.01$ |
| MAP, mmHg | 89 $\pm$ 3 | 89 $\pm$ 3 | 103 $\pm$ 5$^\dagger$ | 92 $\pm$ 4$^\dagger$ | $<0.01$ |
| Diastolic BP, mmHg | 72 $\pm$ 3 | 72 $\pm$ 3 | 83 $\pm$ 5$^\dagger$ | 74 $\pm$ 4$^\dagger$ | $<0.01$ |
| Heart rate, beats/min | 57 $\pm$ 3 | 57 $\pm$ 3 | 54 $\pm$ 4 | 57 $\pm$ 2 | 0.18 |
| RR interval, ms | 1,066 $\pm$ 50 | 1,115 $\pm$ 54 | 1,149 $\pm$ 69 | 1,073 $\pm$ 43 | 0.09 |
| RMSSD, ms | 93 $\pm$ 30 | 134 $\pm$ 34$^*$ | 140 $\pm$ 35$^*$ | 77 $\pm$ 13$^\dagger$ | $<0.05$ |
| FBF, ml/min | 381 $\pm$ 81 | 335 $\pm$ 69 | 366 $\pm$ 82 | 0.78 |
| FVC, ml·min$^{-1}$·mmHg$^{-1}$ | 4.4 $\pm$ 0.9 | 4.3 $\pm$ 0.9 | 3.2 $\pm$ 0.6$^\dagger$ | 4.1 $\pm$ 1.0 | $<0.05$ |
| MSNA total activity, AU | 1,811 $\pm$ 350 | 3,680 $\pm$ 413$^*$ | 5,752 $\pm$ 1,101$^\dagger$ | 2,581 $\pm$ 554$^\dagger$ | $<0.01$ |
| MSNA burst frequency, burst/min | 11 $\pm$ 2 | 17 $\pm$ 3$^*$ | 21 $\pm$ 2$^*$ | 16 $\pm$ 2$^*$ | 0.01 |
| MSNA burst incidence, burst/100 heart beats | 20 $\pm$ 3 | 31 $\pm$ 4$^*$ | 42 $\pm$ 5$^*$ | 28 $\pm$ 5$^*$ | 0.01 |

Values are means $\pm$ SE; $n = 8$ subjects. BP, blood pressure; MAP, mean arterial pressure; RMSSD, root mean square of successive differences of R-R intervals; FBF, femoral blood flow; FVC, femoral vascular conductance; MSNA, muscle sympathetic nerve activity; TGS, trigeminal nerve stimulation. $^*P < 0.05$ vs. rest; $tP < 0.05$ vs. TGS onset. $\dagger P < 0.05$ vs. TGS end.
A summary of the responses evoked during the three protocols is shown in Figs. 2 and 3. Figure 2 shows the percentage change from rest in MAP, HR, FVC, RMSSD, and MSNA for systolic BP, mmHg 120 ± 4 162 ± 5* 152 ± 6† 128 ± 4†‡ 0.01
  MAP, mmHg 87 ± 4 119 ± 4* 111 ± 5† 90 ± 4‡ 0.01
  Diastolic BP, mmHg 70 ± 4 94 ± 4* 87 ± 4† 71 ± 4‡ 0.01
  Heart rate, beats/min 57 ± 2 82 ± 5* 60 ± † 55 ± 2† 0.01
  RR interval, ms 1,068 ± 37 733 ± 46* 1,002 ± 22† 1,102 ± 47‡ 0.01
  RMSSD, ms 114 ± 38 57 ± 13* 113 ± 23† 114 ± 28‡ 0.01
  FBF, ml/min 399 ± 55 338 ± 72 481 ± 114 415 ± 91 0.16
  FVC, ml·min⁻¹·mmHg⁻¹ 4.7 ± 0.7 2.9 ± 0.7* 4.4 ± 1.1† 4.7 ± 1.1† 0.05
  MSNA total activity, AU 2,035 ± 396 5,851 ± 779* 5,424 ± 810‡ 2,820 ± 233‡ 0.01
  MSNA burst frequency, burst/min 12 ± 1 28 ± 3* 25 ± 2† 18 ± † 0.01
  MSNA burst incidence, burst/100 heart beats 21 ± 3 36 ± 5* 41 ± 4* 33 ± 3* 0.05

Values are means ± SE; n = 8. TGS, trigeminal nerve stimulation; IHG, isometric handgrip; PEI, postexercise ischemia. *P < 0.05 vs. rest. †P < 0.05 vs. IHG. ‡P < 0.05 vs. PEI.

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Fig. 1. Original record showing the neural and cardiovascular responses to trigeminal nerve stimulation in one participant. ABP, arterial blood pressure; Resp, respiration; MSNA, muscle sympathetic nerve activity.
the group during trigeminal nerve stimulation alone, isometric handgrip alone, and combined trigeminal nerve stimulation and isometric handgrip. The percentage increase in HR caused by isometric handgrip was slightly but nonsignificantly reduced when trigeminal nerve stimulation was combined with isometric handgrip, and the increase in RMSSD associated with trigeminal nerve stimulation was abolished when trigeminal nerve stimulation was combined with isometric handgrip. Of note, the marked increase in MSNA elicited by either trigeminal nerve stimulation alone or isometric handgrip alone was not significantly greater when the two reflexes were combined.

Figure 3 presents the percentage change from rest for MAP, HR, FVC, RMSSD, and MSNA during trigeminal nerve stimulation alone, during PEI, and combined trigeminal nerve stimulation and PEI. The increase in HR observed during PEI was reduced when this was combined with trigeminal nerve stimulation. Of particular note is that the pronounced increases in MSNA elicited by either trigeminal nerve stimulation alone or PEI alone were not greater during the combination of these two reflexes. Similarly, the increase in MAP and decrease in FVC elicited by each of the reflexes was no different when they were combined.

**DISCUSSION**

In this study we examined the independent and interactive effects of trigeminal nerve stimulation, isometric handgrip, and muscle metaboreflex activation (PEI) on autonomic regulation of heart and peripheral vasculature. The principal new finding of the present investigation is that trigeminal nerve stimulation

| Table 3. Neural cardiovascular values during trigeminal nerve stimulation with isometric handgrip and postexercise ischemia (metaboreflex activation) |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                 | Rest            | IHG + TGS       | PEI + TGS       | Recovery        | P Value         |
| Systolic BP, mmHg               | 122 ± 4         | 161 ± 4*        | 158 ± 6*        | 128 ± 5†‡       | <0.01           |
| MAP, mmHg                       | 90 ± 4          | 120 ± 5*        | 119 ± 6*        | 94 ± 5†‡        | <0.01           |
| Diastolic BP, mmHg              | 73 ± 4          | 96 ± 5*         | 95 ± 6*         | 76 ± 5†‡        | <0.01           |
| Heart rate, beats/min           | 57 ± 3          | 74 ± 5*         | 57 ± 2†         | 56 ± 2†         | <0.01           |
| RR interval, ms                 | 1,068 ± 53      | 844 ± 65*       | 1,059 ± 40†     | 1,082 ± 42†     | <0.01           |
| RMSSD, ms                       | 87 ± 20         | 55 ± 16*        | 131 ± 32*†      | 71 ± 11†‡       | <0.01           |
| FBF, ml/min                     | 403 ± 81        | 318 ± 122       | 442 ± 123       | 403 ± 94        | 0.42            |
| FVC, ml·min⁻¹·mmHg⁻¹             | 4.6 ± 1.0       | 2.8 ± 1.1*      | 3.7 ± 1.0       | 4.4 ± 1.1†‡     | <0.05           |
| MSNA total activity, AU         | 1,920 ± 335     | 5,948 ± 759*    | 6,932 ± 1,131*  | 2,717 ± 408‡‡   | <0.01           |
| MSNA burst frequency, burst/min | 12 ± 2          | 23 ± 2*         | 27 ± 2*         | 16 ± 2*‡‡       | <0.01           |
| MSNA burst incidence, burst/100 heart beats | 21 ± 3 | 34 ± 5* | 46 ± 4*† | 29 ± 3† | <0.01 |

Values are means ± SE; n = 8. *P < 0.05 vs. rest. †P < 0.05 vs. IHG + TGS. ‡P < 0.05 vs. PEI + TGS.
Facial cooling evoked a rapid and robust increase in MSNA that was similar in magnitude when undertaken separately, or in combination with isometric handgrip or muscle metaboreflex activation. Thus, with respect to MSNA, the intensity of excitatory input from the trigeminal nerve achieved in this study did not show algebraic summation with an input from metabolically sensitive skeletal muscle afferents. It appears the results demonstrate synaptic occlusion, suggesting there is a high degree of convergence of quite different receptor pathways on the neural circuits controlling cardiovascular neurons.

The pons receives inputs from the ophthalmic and maxillary divisions of the trigeminal nerve via the trigeminal ganglion. Whereas descending inputs from suprabulbar regions may exert some modulatory influence, the neural circuitry within the medulla oblongata has been identified as the principal regulator of the autonomic responses to diving in experimental mammals (4, 34). In humans, facial immersion in cold water evokes a pronounced increase in MSNA, peripheral vascular resistance, and blood pressure and a decrease in HR (15, 20). However, along with stimulation of the trigeminal nerve, the apnea that accompanies facial water immersion is also likely to contribute to the autonomic cardiovascular responses elicited in experimental mammals (11, 21, 29). To circumvent this in the present study, facial cooling was employed while respiratory frequency and tidal volume were guided at a predetermined normal rate and depth. We observed that this maneuver robustly increases MSNA, FVC, and blood pressure. Such sympathetic and pressor responses are comparable to the magnitude of the increases observed with facial immersion (15), perhaps indicating the importance to the MSNA response of trigeminal afferent linkage to cardiovascular centers rather than respiratory ones in humans.

Moderate-intensity isometric handgrip and PEI were both observed to evoke robust increases in MSNA, as has previously been described (30, 33). The activation of metabolically sensitive skeletal muscle afferents (e.g., muscle metaboreflex) has been identified as principally responsible (rather than central command) for the sympathoexcitation evoked during isometric handgrip (46–48). The very similar elevation in MSNA during isometric handgrip and during PEI, where the contribution of the muscle metaboreflex to the sympathoexcitatory to isometric handgrip is isolated, is further evidence of this. The metabolically sensitive skeletal muscle afferents travel via the dorsal horn of the spinal cord to provide feedback to brain stem cardiovascular areas (e.g., nucleus of the solitary tract, rostral ventrolateral medulla) (38). Notably, the magnitude of the sympathetic response to trigeminal nerve stimulation when performed in combination with isometric handgrip or isolated muscle metaboreflex activation was not statistically different from that observed when it was performed separately. This suggests that afferent inputs from the trigeminal nerve and group III and IV fibers of the skeletal muscle have considerable convergence on the muscle vasomotor pools of neurons within the central nervous system (e.g., medulla oblongata) and exhibit synaptic occlusion. It may be argued that the similar MSNA responses to trigeminal nerve stimulation, isometric handgrip, and muscle metaboreflex activation are indicative of a “ceiling effect.” Schobel et al. (40) noted that the reflex MSNA response evoked by immersion of a hand in ice water (cold pressor test) was inversely related to the resting MSNA, suggesting that a higher baseline MSNA diminishes the potential for further sympathoexcitation. Similarly, when MSNA is heightened during PEI, the capacity to increase it further with trigeminal nerve stimulation may be diminished. However, this is unlikely...
to account for the present observations, since isometric handgrip was performed at 25% MVC, and others have shown that the MSNA response to isometric handgrip and a following period of PEI is graded according to the strength of the contraction (39). Synaptic occlusion may have important survival implications for the diving animal where there is a need to prevent excessive increases in cardiac work and blood pressure caused by enhanced peripheral resistance. In regards to the heart, a concomitant increase in cardiac sympathetic and parasympathetic activity has been linked with an increased frequency of arrhythmic events during diving (36, 41). It is speculated that, if this proarrhythmic milieu or autonomic conflict occurred during water immersion, it would be exacerbated by the exercise-induced cardiac sympathetic activation (42, 44). Our data suggest that cardiac sympathetic activity is not increased, since the muscle sympathoexcitatory response to trigeminal stimulation is not enhanced when undertaken with exercise. In fact, the combination of isometric handgrip and trigeminal stimulation tended to evoke a smaller increase in HR than during isometric handgrip alone, although these responses did not reach statistical significance. Nevertheless, the lack of a greater increase in HR during combined isometric handgrip and trigeminal stimulation may be indicative of there being no heightening of the underlying cardiac sympathetic nerve activity. This would be in accord with studies in urethane-anaesthetized rabbits that have reported that nasopharyngeal receptor (trigeminal) stimulation not only increases cardiac vagal tone but also decreases cardiac sympathetic nerve activity while renal sympathetic nerve activity increases (37).

We undertook direct recordings of central sympathetic outflow to the skeletal muscle vasculature, which under some circumstances exhibits a significant linear correlation with cardiac norepinephrine spillover, the “gold standard” measure of cardiac sympathetic nerve activity (28), but whether cardiac sympathetic nerve activity was similarly positively correlated in this study was not feasible for us to directly assess. Thus, it remains to be determined more directly whether inputs from the trigeminal nerves and metabolically sensitive skeletal muscle afferents similarly fail to summate algebraically in determining cardiac sympathetic nerve activity in humans.

Simulation of the diving response in humans has been shown to evoke an atropine-sensitive bradycardia (16, 26). Furthermore, trigeminal nerve stimulation by face immersion in water has been shown to increase respiratory sinus arrhythmia and cardiac baroreflex sensitivity in humans (14) and cardiac baroreflex sensitivity in anesthetized seals (7). We observed a significant increase in RMSSD during trigeminal nerve stimulation via facial cooling, indicative of an increase in cardiac parasympathetic activity during this maneuver (43a). However, the magnitude of the reduction in HR (and increase in R-R interval) was smaller than that noted previously during facial water immersion or cold pads on the face and did not reach statistical significance for the group ($P = 0.18$ and $0.09$, respectively) (2, 8, 15, 26). There are a number of possibilities that may account for this observation. Six of the eight subjects exhibited a reduction in HR with trigeminal nerve stimulation, and thus the potential for a type II error is acknowledged. The observed partial eta squared (0.261) indicates that 22 subjects were required to detect an increase in R-R interval with trigeminal stimulation, but, given that the focus of the present study was on MSNA, we did not feel that this was a reasonable number to obtain. The smaller HR response may in part be attributable to our participant’s voluntarily breathing at their normal rate, since apnea is considered important for the full expression of the bradycardic response to diving (5, 8, 11, 21). Another possibility is resting cardiac parasympathetic activity was enhanced in this young recreationally active group who were studied in a supine position, and this may have diminished the potential for further increases in cardiac parasympathetic activity in some individuals (1, 43a).

During isometric handgrip, HR increases and cardiac parasympathetic activity decreases as a consequence of central command and skeletal muscle afferent feedback (18). We observed that HR was similarly increased and RMSSD was similarly decreased in the isometric handgrip trial and isometric handgrip combined with trigeminal nerve stimulation trial. Thus we suspect that the ability of trigeminal nerve stimulation to increase cardiac parasympathetic activity during isometric handgrip was prevented by the inhibitory effects of central command and/or skeletal muscle afferent feedback on cardiac parasympathetic activity (2, 43). During PEI following moderate-intensity isometric handgrip, blood pressure remains elevated while HR returns to the resting level (18, 30). At this time both parasympathetic and sympathetic activity to the heart are elevated because of loss of inhibitory influences of central command and muscle mechanoreceptors and/or an increase in the cardiac component of the baroreflex. Thus the elevated cardiac parasympathetic activity prevails and prevents a notable HR increase (17, 18).

Heath and Downey (25) reported a consistent cardiovascular response to multiple trials of facial cooling, although this has not been a universal finding (8). To avoid the potential for a decreased response to the repetition of the cold face, exposure trials were counterbalanced and separated by $\approx 20$ min. To minimize variations in ice pack, position was placed each time by a single investigator. Along with the determination of spontaneous cardiac baroreflex sensitivity, we attempted to assess arterial baroreflex control of MSNA as previously described (27). However, although significant relationships between diastolic blood pressure and MSNA were found in all subjects at rest, this was not the case during trigeminal nerve stimulation, indicating that this analysis was unsuitable to determine arterial baroreflex control of MSNA. Selections of data of differing lengths were used to compare some time periods (e.g., 3 min at rest and 15 s during end-handgrip); however, our major conclusions are based on data obtained over equivalent lengths (i.e., comparison of the responses to trigeminal stimulation, handgrip or PEI alone, and in combination; Figs. 2 and 3). Finally, our studies were undertaken in young healthy men; therefore, caution is required in extrapolating our findings to older subjects or patient populations in whom alterations in reflex autonomic control have been indicated, such as hypertension or heart failure.

In summary we observed that trigeminal nerve stimulation by facial cooling evokes a robust increase in MSNA, MAP, and peripheral vasoconstriction that is similar in magnitude when undertaken independently or in combination with isometric handgrip or muscle metaboreflex activation, suggesting that these separate excitatory pathways to the central nervous system have a high degree of convergence on the neural circuits controlling MSNA and perhaps more generally. Such synaptic occlusion may ensure that cardiac work is optimized by pre-
venting too great an increase in peripheral vascular resistance and blood pressure during exercise when submerged.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


