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Sympathetic nerve activity and simulated diving in healthy humans

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ABSTRACT

The goal of our study was to develop a simple and practical method for simulating diving in humans using facial cold exposure and apnea stimuli to measure neural and circulatory responses during the stimulated diving reflex. We hypothesized that responses to simultaneous facial cold exposure and apnea (simulated diving) would be synergistic, exceeding the sum of responses to individual stimuli. We studied 56 volunteers (24 female and 32 male), average age of 39 years. All subjects were healthy, free of cardiovascular and other diseases, and on no medications. Although muscle sympathetic nerve activity (MSNA), blood pressure, and vascular resistance increased markedly during both early and late phases of simulated diving, significant reductions in heart rate were observed only during the late phase. Total MSNA during simulated diving was greater than combined MSNA responses to the individual stimuli. We found that simulated diving is a powerful stimulus to sympathetic nerve traffic with significant bradycardia evident in the late phase of diving and eliciting synergistic sympathetic and parasympathetic responses. Our data provide insight into autonomic triggers that could help explain catastrophic cardiovascular events that may occur during asphyxia or swimming, such as in patients with obstructive sleep apnea or congenital long QT syndrome.

1. Introduction

The diving reflex has been demonstrated in animal studies to be a powerful autonomic stimulus (Andersen, 1966; Blix and Folkow, 1983; Hong, 1989). Unlike other stresses that accentuate either sympathetic or parasympathetic outflow, with reciprocal inhibition of one or the other, diving stimulates simultaneously both of these major components of the autonomic nervous system in birds and mammals (Butler and Jones, 1997; McPhail and Jones, 1999; Davis et al., 2004). Diving reflex responses include vagally-mediated bradycardia in humans (Asmussen and Kristiansson, 1968), simultaneous with diffuse and marked increases in sympathetic-mediated peripheral vasoconstriction in several vascular beds, for the purpose of maintaining circulation to vital organs such as the brain and heart in humans (Elsner et al., 1971; Leuenberger et al., 2001). The primary objective of this reflex response is to conserve oxygen in order to enable underwater exploration by birds and mammals (Butler and Jones, 1997) while maintaining neural and cardiac integrity.

The role of the diving reflex in circulatory homeostasis in humans is poorly understood. However, it has been implicated in drowning and catastrophic cardiovascular events, particularly in patients with congenital long QT syndrome (LQTS) (Ackerman et al., 1999; Batra and Silka, 2002). Enhanced sympathetic activity is an established cause of ventricular tachyarrhythmia (Vassalle et al., 1970). Diving-induced bradycardia is associated with ventricular ectopic beats in healthy humans (Smith et al., 1997). Vagal bradycardia and simultaneous sympathetic activation during the diving reflex (Paton et al., 2006) may be a potential trigger for arrhythmias and possibly sudden death in patients with vulnerable substrates, such as LQTS in general and type 1 LQTS in particular (Ackerman et al., 1999). Activation of the diving reflex also may occur during apneic episodes in patients with obstructive sleep apnea (Rial et al., 2000), sometimes resulting in pacemaker placement, and has been implicated in increased cardiovascular mortality. Although the diving reflex has been studied extensively in animals for more than a century, human studies are few, in part due to the complexity of appropriate stimuli. Immersion of the face in water is an important component of the stimulus (Anderson, 1963; Dykes, 1974; Schuitema and Holm, 1988), and face immersion alone increases vagal activity (Hayashi et al., 1997). Therefore, in the laboratory setting, simulated "diving" and "facial cold" have been introduced to study the diving reflex in humans (Whayne and Killip, 1967; Heistad et al., 1968; Heath and Downey, 1990).

Although less marked than in some animals, bradycardia due to increased vagal activity during simulated diving has been observed in humans (Craig, 1963; Kawakami et al., 1967; Leuenberger et al., 2001; Andersson et al., 2004; Foster and Sheel, 2005). However, little is known about the sympathetic neural responses associated with simulated diving. Microneurography is a unique method for direct measurement

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of sympathetic nerve outflow in humans (Shamsuzzaman and Somers, 2003; Vallbo et al., 2004; Mano et al., 2006; Wallin and Charkoudian, 2007). To our knowledge, the only study that systematically evaluated the diving reflex and its effect on muscle sympathetic nerve activity (MSNA) in humans noted marked increases in SNA accompanied by peripheral vasoconstriction and increased blood pressure (BP) (Fagius and Sundlof, 1986). How these changes compare to the effects of apnea alone or facial cold exposure alone was not studied. Furthermore, subjects were studied in the prone position with only 12 s of facial immersion, and bradycardia as a key component of the diving response was not statistically significant (Fagius and Sundlof, 1986). Short stimulus duration may have affected the measurements.

The goal of our study was to develop a simple and practical method for simulating diving in humans using facial cold exposure and apnea stimuli to measure neural and circulatory responses during the stimulated diving reflex. We hypothesized that neural circulatory responses to simulated diving induced by both facial cold and apnea are synergistic, exceeding the sum of the individual responses to either facial cold or apnea.

2. Methods

2.1. Subjects

We studied 56 healthy volunteers (24 female and 32 male), average age of 39 ± 14 years with body mass index (BMI) of 26 ± 4. All female volunteers were pre-menopausal and not menstruating during the study. All subjects were healthy, free of cardiovascular and other diseases, on no medications, and were asked to avoid exercise and caffeinated beverages for at least 24 h before the study. Informed written consent was obtained from all subjects. The study was approved by the Mayo Clinic's Institutional Review Board.

2.2. Measurements and facial cold exposure material

A 12-lead electrocardiogram (ECG) was recorded continuously by ECG Bioamplifier (Gould Electronics) and respiration was measured using a thoracic belt (Pneumotrace, Gould Electronics). Beat-to-beat changes in BP were recorded continuously by a Finapres (Ohmeda, Louisville, CO). BP also was measured every minute from the brachial artery with an automatic sphygmomanometer (Dinamap, Critikon Inc.). Blood flow was measured from the left calf by venous occlusion plethysmography (EC4, Hokanson) in 11 subjects. Multiunit efferent intraneural recordings of MSNA to the skeletal muscle blood vessels were obtained from the right peroneal nerve posterior to the head of the fibula, using microneurography (Shamsuzzaman and Somers, 2003).

For facial cold exposure, a specially constructed water-proof polyethylene bag with a central opening for the nose to maintain spontaneous nasal breathing was used. The bag, filled with ice and water, was applied to cover the entire face except the nose for facial cold. Before applying the bag, two pieces of shielding gauze were applied over closed eyelids to protect them from cold and pressure, thus minimizing the possibility of activating the oculocardiac reflex.

2.3. Protocol

All subjects were studied in the supine position. Measurements were made in a quiet, air-conditioned room with 22–25 °C ambient temperature. After 15 min of recovery following instrumentation, technically high-quality recordings of MSNA (signal to noise ratio ≥ 3:1) together with measurements of BP, heart rate (HR), respiration, and calf blood flow were obtained while the subject rested in the supine horizontal position in an undisturbed environment. After an acceptable recording of MSNA was established, subjects rested supine for 15 min, during which MSNA and all cardiovascular measures were recorded continuously. At the end of baseline recordings, subjects were asked to hold their breath after full expiration (end-expiratory apnea) for maximum possible duration. Subjects then rested for a 10-minute period to permit hemodynamics and MSNA to return to baseline levels. A plastic bag was filled with approximately 50% ice and 50% water. The plastic bag was stored in a bucket containing a mixture of ice and water to keep it wet. A towel was placed around the subject's head and neck to protect from dripping water, before application of the facial cold. The cold and wet plastic bag was applied to the face for 1 min for facial cold alone, after which the subject was asked to hold his or her breath after full expiration for a maximum duration for apnea while facial cold continued (simulated diving). Blood flow was measured from the left calf for 2 min at the end of the baseline resting period and continuously during individual apnea and facial cold exposure, as well as during simulated diving.

2.4. Data storage and analysis

The data were digitized on a computer and simultaneously stored on digital audio tape. Baseline data were averaged for each minute. The duration of apnea and thus simulated diving was different between subjects. Therefore, apnea, facial cold, and simulated diving periods were divided into halves, an early phase and a late phase, and results were expressed as per minute. HR was calculated from the ECG by counting the number of R-waves per minute. Systolic, diastolic, and mean BP were calculated from the Finapres BP wave after careful identification of the peak and trough. Blood flow was measured by venous occlusion plethysmography as previously described (Kato et al., 2000). Vascular resistance (VR) was calculated by dividing mean arterial pressure (MAP) by calf blood flow and is expressed in arbitrary units.

Sympathetic bursts were identified and counted manually after careful inspection of the mean voltage neurogram and expressed as MSNA burst frequency (bursts/min) and burst incidence (bursts/100 heart beats). MSNA burst amplitude was determined, and total MSNA was calculated as burst rate multiplied by mean burst amplitude and expressed as arbitrary units per minute. Neural circulatory changes during apnea, facial cold, and simulated diving were calculated as both absolute changes and percentage changes from the baseline resting period, considering baseline activity as 100%.

2.5. Statistical analysis

Data are expressed as means ± standard deviation. Neural and circulatory responses to apnea, face cold, and simulated diving during early and late phases were analyzed by ANOVA with post-hoc comparison between the stimuli using Student's *t*-test. In all tests, a value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of apnea

Prolonged apnea was associated with late potentiation of MSNA. Original recordings of ECG, BP, respiration, and integrated MSNA during baseline resting conditions and during apnea in a single subject are shown in Fig. 1. Significant increases in MSNA, VR, and BP were observed only during the late phase of apnea (Fig. 4).

3.2. Effects of facial cold exposure

Original recordings of ECG, BP, respiration, and integrated MSNA during baseline resting, facial cold, and simulated diving in the same subject are shown in Fig. 1. Facial cold for 1 min was associated with increased MSNA, VR, and BP during both early and late phases (Figs. 2–4). However, facial cold had no effect on breathing rate.