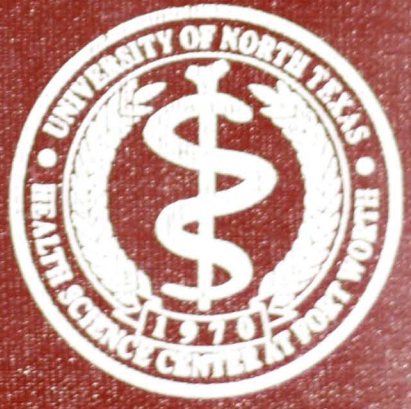
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Muenter Swift, Nicolette, Mechanisms of Post-Apneic Sympathoinhibition in Humans. Doctor of Philosophy (Biomedical Sciences), August, 2002, 110 pp., 14 figures, references.

Apnea is accompanied by a concomitant rise in arterial pressure and muscle sympathetic nerve activity (MSNA), the latter primarily due to chemoreflex stimulation and possibly the lack of sympathoinhibitory input from pulmonary stretch receptors. The progressive sympathoexcitation during apnea suggests a possible overriding of arterial baroreflex sympathoinhibitory input to sympathoregulatory centers by apnea-induced sympathoexcitatory mechanisms. Nevertheless, it is unknown whether apnea attenuates baroreflex control of MSNA. Apnea termination is accompanied by a profound and immediate sympathoinhibition, the mechanisms of which are unclear; however, potential mediators include normalization of blood gases (i.e., chemoreflex unloading), the lung inflation reflex, and arterial baroreflex stimulation. Therefore, the purpose of the current studies was to: i) determine the contribution of chemoreflex unloading to post-apneic sympathoinhibition, ii) determine the contribution of the lung inflation reflex to post-apneic sympathoinhibition, and iii) determine whether carotid baroreflex control of MSNA is altered by apnea and its termination. The first study compared MSNA during post-apneic administration of room air versus a gas mixture designed to maintain the subjects' end-apneic alveolar gas levels. Regardless of post-apneic gas administration, post-apneic MSNA was at or below baseline pre-apneic levels; thus, chemoreflex

unloading does not contribute importantly to post-apneic sympathoinhibition. Furthermore, quantification of post-apneic MSNA associated only with the low lung volume phase of respiration, when sympathoinhibitory input from the lung inflation reflex is minimal, demonstrated that post-apneic sympathoinhibition persists even during the low lung volume phase of respiration. Therefore, the lung inflation reflex does not appear to be the primary mediator of post-apneic sympathoinhibition. The second study utilized neck suction (NS) and neck pressure (NP) to assess carotid baroreflex function during and following apnea. The sympathoinhibitory response to –60 Torr NS was maintained throughout apnea; conversely, the sympathoexcitatory response to +30 Torr NP was attenuated for nearly one minute post-apnea. Thus, carotid baroreflex control of MSNA is not altered by apnea but is transiently attenuated by apnea termination. We propose that the carotid baroreflex-MSNA function curve resets rightward and upward during apnea. Return of the function curve to baseline upon apnea termination may partly explain the reduced MSNA response to NP post-apnea.

MECHANISMS OF POST-APNEIC SYMPATHOINHIBITION

IN HUMANS

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MECHANISMS OF POST-APNEIC SYMPATHOINHIBITION IN HUMANS

DISSERTATION

Presented to the Graduate Council of the

Graduate School of Biomedical Sciences

University of North Texas Health Science Center at Fort Worth

in Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

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Fort Worth, Texas

August 2002

ACKNOWLEDGEMENTS

I extend my gratitude and thanks to my mentor, Dr. Michael L. Smith. He has shown faith in me from the beginning, when he accepted me into his laboratory knowing little about me. He has provided excellent guidance throughout this project and has always been most encouraging. I also thank Dr. Peter B. Raven for his support and guidance, as well as my other committee members, Dr. Robert Mallet, Dr. David Barker, Dr. John Burk, and Dr. Walter McConathy. The other members of Dr. Smith's and Dr. Raven's laboratories have been invaluable to me, providing technical help, patient teaching, advice, and friendship over the years: Dr. Don Watenpaugh, Dr. Stephen Wasmund, Wendy Wasmund, Michael Cutler, Dr. Robert Carter III, Dr. Paul Fadel, Dr. Shigehiko Ogoh, David Keller, and Selena Godoy. To them I am very thankful and indebted.

I also extend my deepest gratitude and appreciation to my family; they have guided me, supported me, believed in me, and been proud of me all throughout life. I must especially thank my father, who has constantly encouraged me to reach my goals and has always insured that I have the opportunity and means to pursue those goals. His enthusiasm for everything in life is a constant inspiration for me. I also thank my husband Jon, for his eternal patience, understanding, and support.

This project was supported by a grant to Dr. Michael L. Smith from the National Institutes of Health / NIH Grant #HL 49266. My education and training was supported

by the Department of Integrative Physiology and the Graduate School of Biomedical Sciences of the University of North Texas Health Science Center at Fort Worth. Additional funding was provided by a fellowship from the Graduate Student Researchers Program of the National Aeronautics and Space Administration of the United States of America / NASA Grant #NGT5-50179 and the Graduate Research Enhancement and Training Program of the University of North Texas Health Science Center. The following original articles, published abstracts, and presentations are the results of this research training.

Original Articles

Muenter Swift N, Watenpaugh DW, Wasmund SL, Wasmund WL, Smith ML. Mechanisms of the sympathoinhibition associated with the termination of voluntary apnea. In submission.

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LIST OF ABBREVIATIONS

A1-A3	first, second, and last third of apnea
ANOVA	analysis of variance
BP	arterial blood pressure
CBR-MSNA	carotid baroreflex-muscle sympathetic nerve activity
CO_2	carbon dioxide
ECG	electrocardiogram
HB	heartbeat
Insp Flow	inspiratory airflow
MAP	mean arterial pressure
MSNA	muscle sympathetic nerve activity
NCP	neck chamber pressure
NP	neck pressure
NS	neck suction
NTS	nucleus tractus solitarius
O_2	oxygen
%	percent
R1-R6	first through sixth consecutive ten second time segments post-apnea

s	second(s)
Sa_{O_2}	arterial oxygen saturation
SE	standard error
V_T	tidal volume

CHAPTER I

INTRODUCTION

The temporary cessation of respiration, or apnea, whether voluntarily performed in conscious individuals or occurring spontaneously in sleep apnea patients, leads to the development of progressive hypoxemia and hypercapnia, elevated muscle sympathetic nerve activity (MSNA), and elevated arterial blood pressure (24, 25, 32, 35, 41, 54, 55, 57, 69). During apnea, sympathoregulatory centers in the medulla are receiving conflicting input from a variety of reflexes. Combined hypoxemia and hypercapnia stimulate the chemoreflexes to synergistically increase MSNA (56, 59). Lack of stimulation to the pulmonary stretch afferents, which are inhibitory to MSNA (20, 51, 59, 60, 63, 77), may also contribute to sympathetic activation during apnea. Elevated arterial pressure is inhibitory to MSNA via the arterial baroreflexes (6, 10, 46, 61, 73, 74). However, in spite of elevated arterial pressure, apneic events lead to profound increases in MSNA (24, 25, 32, 35, 41, 55, 57), suggesting that the sympathoinhibitory inputs from baroreceptors are overridden by apnea-induced sympathoexcitatory mechanisms.

Apnea termination is accompanied by a profound and immediate sympathoinhibition (24, 25, 35, 62, 75). The mechanism(s) for this sympathoinhibition is unknown; however, possible contributors include normalization of blood gases (i.e., chemoreflex "unloading"), activation of pulmonary stretch afferents via lung inflation,

and negative feedback from arterial baroreflexes due to elevated arterial blood pressure. However, the importance and relative contribution of each of these mechanisms to post-apneic sympathoinhibition is unclear. Therefore, the purpose of these dissertation studies is: 1) to determine the importance of chemoreflex unloading to post-apneic sympathoinhibition; 2) to determine the role of lung inflation in post-apneic sympathoinhibition; and 3) to determine whether carotid baroreflex control of MSNA is altered by apnea and its termination.

Review of Related Literature

The condition of apnea and its termination involve complex interactions among several reflexes. Included among these are the lung inflation reflex, peripheral and central chemoreflexes, and arterial baroreflexes. It is important to consider not only the reflexes individually, but the interactions among them as well.

Lung Inflation Reflex

Pulmonary stretch receptors are mechanoreceptors located in the smooth muscle layer of the airways of the lungs, and they are stimulated by lung inflation (7, 28, 76). Animal studies have shown that pulmonary stretch receptor afferents, traveling via the vagus nerve, fire almost exclusively during the inspiratory phase of respiration (1, 3). Gerber and Polosa first demonstrated that stimulation of pulmonary stretch receptor afferents, either by lung inflation or direct electrical stimulation, decreased preganglionic

sympathetic neuron firing in anesthetized cats (20). Lung inflation has since been shown to decrease sympathetic activity, systemic vascular resistance, and arterial pressure in other animal species (4, 9, 33, 77).

In humans, MSNA fluctuates with respiration such that neural activity decreases during inspiration and increases during expiration (10, 15, 23, 51, 52, 63, 68), reaching its nadir and peak at end-inspiration and end-expiration, respectively (10, 15, 52, 63). This results in the majority of all MSNA occurring during the low lung volume (ie., last half of expiration and first half of inspiration) phase of normal respiration when basal MSNA is low to moderate (39, 52). This respiratory modulation of MSNA becomes more apparent (ie., increased sympathoinhibition and sympathoexcitation during inspiration and expiration, respectively) when tidal volumes (V_T) are increased above normal resting values (51, 52, 63, 68). In addition, respiratory modulation of MSNA is rate sensitive, as increasing the inspiratory rate causes sympathoinhibition to occur earlier in inspiration (ie., at a smaller volume increase) and vice versa (51). However, it is important to note that increasing V_T alone or in combination with increased respiratory rate, whether controlled for end-tidal CO_2 or not, does not alter total MSNA, indicating that increased sympathoinhibition during high lung volume is balanced by increased sympathoexcitation during low lung volume (51, 52, 63, 68). Also, this respiratory modulation of MSNA is less apparent when basal MSNA is very high (for example, in conditions such as heart failure): MSNA bursts occur throughout the respiratory cycle.

In addition to reducing sympathetic traffic, lung inflation has also been shown to inhibit cardiac vagal efferent nerve traffic in animals, producing a reflex tachycardia (2,

3, 29-31). In humans, R-R interval decreases with inspiration and increases with expiration (13, 15, 63), and this sinus arrhythmia increases in magnitude as V_T increases (68).

Chemoreflexes

The primary peripheral chemoreceptors are located in the carotid body, which receives its blood supply from the common carotid artery and thus arterial blood gas concentrations can be carefully monitored (44). Peripheral chemoreceptors respond principally to hypoxia while central chemoreceptors, located in the medulla, respond principally to hypercapnia (5, 19, 38, 44, 72). Carotid chemoreceptor afferents travel to the brainstem via the glossopharyngeal nerve and synapse with nucleus tractus solitarius neurons (44, 71), which in turn modulate sympathetic and vagal neural outflow.

Chemoreceptor activation by either hypoxia or hypercapnia increases MSNA, both in animals (6, 21, 22) and in humans (24, 34, 42, 48, 49, 59-61), and combined hypoxia/hypercapnia synergistically increases MSNA (56, 58, 59). In addition, chemoreceptor activation increases ventilation and heart rate (24, 26, 42, 48, 49, 59-61, 65, 78). Thus, this synergistic interaction is an important mechanism of sympathoexcitation during sleep apnea events.

Carotid Baroreflex

The carotid and aortic arterial baroreflexes importantly monitor and regulate arterial blood pressure on a beat-to-beat basis, responding to alterations in pressure by

reflexively increasing or decreasing sympathetic and parasympathetic tone. Carotid and aortic baroreceptor endings are mechano-sensors, sensitive to stretch, located in the carotid sinus and aortic arch with their cell bodies located in the petrosal and nodose ganglia, respectively (44, 71). Carotid and aortic baroreceptor afferents travel via the glossopharyngeal and vagus nerves, respectively, and converge on neurons of the nucleus tractus solitarius (NTS) in the medulla (44, 71). These neurons in turn project to other areas of the brainstem involved in control of sympathetic and parasympathetic outflow. NTS neurons project to neurons of the caudal ventrolateral medulla, which in turn project inhibitory neurons to the rostral ventrolateral medulla (71). Sympathoexcitatory neurons of the rostral ventrolateral medulla send preganglionic efferents, via the intermediolateral column of the spinal cord, to the sympathetic ganglia where they synapse with postganglionic sympathetic neurons (71). NTS neurons also have parallel excitatory projections to preganglionic parasympathetic neurons in the nucleus ambiguus, the dorsal nucleus of the vagus, and the rostral ventromedial medulla (71). Thus, increases in arterial pressure will stimulate afferent baroreceptor input to the NTS, resulting in a reflexive decrease in sympathetic outflow and increase in parasympathetic outflow. The decrease in sympathetic tone leads to vasodilation and a decrease in systemic vascular resistance, while the increase in parasympathetic tone slows heart rate and decreases cardiac output; thus, arterial pressure is returned toward its original value. Conversely, decreases in arterial pressure will reduce baroreceptor afferent firing, reflexively increasing sympathetic outflow and decreasing parasympathetic outflow.

One of the commonly utilized methods for assessing carotid baroreflex function is the application of suction and/or pressure to an airtight chamber enclosing the neck, thus altering carotid transmural pressure and baroreceptor afferent neural activity. This method was first described by Ernsting and Parry (16) and later simplified by Eckberg and colleagues (11), who introduced a smaller, inexpensive, one-size-fits-most neck collar which allowed for a rapid development (~100 ms) of the desired level of neck chamber pressure. This enabled investigators to time the delivery of the neck suction (NS) and neck pressure (NP) pulses within the cardiac cycle, which is important because this timing affects the measured response (11, 12, 36).

There are several advantages to using the NS/NP system to assess carotid baroreflex function. Carotid baroreflex function can be tested separately from aortic baroreflex function, unlike with drug infusions in which input to both types of baroreceptors are simultaneously altered. Application of NS/NP also avoids nonreflex-mediated drug effects. Additionally, it has been shown that NS/NP does not stimulate chemoreceptors by affecting carotid or cerebral blood flow (11, 37).

The carotid sinus pressure-MSNA relation is described by an inverse sigmoid function, with normal human subjects in the supine position operating near the onset pressure for sympathoexcitation (46). Thus, responses to NS/NP are asymmetric, with NP-induced sympathoexcitation greater than sympathoinhibition produced by NS of an equivalent magnitude; in other words, in healthy supine subjects, the carotid baroreceptors are better poised to counteract a hypotensive rather than hypertensive change in pressure, at least in regards to the sympathetic arm of the reflex (46, 74).

However, the reflex can rapidly reset the control of MSNA, as shown during dynamic exercise (17). This may also play a role in the MSNA response during apnea. In regards to the cardiac arm of the reflex, the carotid sinus pressure-R-R interval relation is a sigmoid function curve, with healthy individuals operating in the linear portion of the function curve (46).

Interaction between Lung Inflation Reflex and Chemoreflex

During chemoreflex-induced hyperventilation, the within-breath modulation of MSNA is preserved in spite of an increase in total MSNA (52, 56). In other words, the lung inflation reflex retains its ability to inhibit MSNA in the face of chemoreflex-induced sympathoexcitation. Thus, chemoreflex-induced sympathoexcitation is markedly greater in the absence of lung inflation, as during apnea (24, 59, 60). The presence of respiration also affects the cardiac response to chemoreflex stimulation: tachycardia is observed when chemoreflex stimulation is accompanied by respiration, while bradycardia is observed when chemoreflex stimulation is accompanied by apnea (79).

Interaction between Lung Inflation Reflex and Carotid Baroreflex

It has been observed both in animals and in humans that the respiratory cycle affects the sympathetic response to carotid baroreflex stimuli (15, 53). Eckberg and colleagues demonstrated that a hypotensive stimulus of +30 Torr NP produced greater sympathoexcitation during expiration, when sympathoinhibitory input from the lung

inflation reflex would not have been present (15). St. Croix *et al.* analyzed respiratory fluctuations in diastolic pressure and MSNA, and found that for a given change in pressure, MSNA was less at higher lung volumes than at lower lung volumes, and attributed this to the lung inflation reflex (63). Thus, it appears that the lung inflation reflex is able to modulate MSNA responses to baroreflex input.

Similar results have been observed in the cardiac arm of the reflex as well. Animal studies have shown that lung inflation reduces baroreflex-induced bradycardia (8, 18). Studies in humans agree, finding a decreased bradycardic response to NS during inspiration versus expiration (13, 64). However, both studies also found that this modulation of carotid baroreflex-cardiac responsiveness by lung inflation could be overcome if the baroreflex stimulus was great enough (i.e., -40 Torr NS in one study, -60 Torr NS in the other study) (13, 64). Thus, respiratory phase affects the cardiac responsiveness to moderate but not strong carotid baroreflex stimulation.

Interaction between Chemoreflex and Baroreflex

Carotid chemoreceptor and baroreceptor afferents both converge upon neurons of the NTS (44, 71). It is therefore not surprising that processing of input from one reflex is affected by input from the other. Baroreflex activation attenuates the MSNA and vasoconstrictor responses to peripheral chemoreflex stimulation in dogs (27, 40). Attenuation of the peripheral chemoreflex-induced increase in MSNA and heart rate by baroreflex activation has also been observed in humans (61). Conversely, peripheral chemoreflex stimulation has been shown to reduce cardiac sensitivity to baroreflex

activation in humans (78). These data demonstrate the ability of the baroreflex to modulate responses to chemoreflex stimulation, and vice versa.

Apnea: the Ultimate Interaction

During apnea, MSNA increases progressively and dramatically (24, 25, 35, 41, 43, 57, 59, 60, 62, 75), and chemoreflex activation has been shown to contribute importantly to this sympathoexcitation (24, 35, 41, 57). The removal of the sympathoinhibitory influence of lung inflation is also likely contributing to the apnea-induced sympathoexcitation (24, 59, 60). Arterial pressure increases throughout apnea in response to the sympathoexcitation (24, 25, 32, 35, 41, 47, 62, 70, 75); this rise in pressure would normally be expected to inhibit MSNA via the arterial baroreflexes (6, 10, 46, 50, 61, 73, 74). The concurrent rise in arterial pressure and MSNA indicate that input from the arterial baroreceptors is overridden during apnea by sympathoexcitatory mechanisms.

Apnea termination is accompanied by an immediate and near complete sympathoinhibition (24, 25, 35, 62, 75). Several conditions of post-apneic recovery may be contributing to the sympathoinhibition. First, progressive normalization of blood gases would remove chemoreflex stimulation (i.e., chemoreflex unloading), removing the synergistic sympathoexcitatory input from the peripheral and central chemoreceptors. Considering the important role that chemoreflex stimulation plays in the apnea-induced sympathoexcitation (24, 35, 41, 57), it follows that the post-apneic sympathoinhibition is due, in part, to the normalization of blood gases. However, the abruptness and magnitude

of the sympathoinhibition suggest that perhaps other mechanisms are responsible. Our laboratory previously reported that the post-apneic sympathoinhibition occurs before Sa_{O_2} levels return to baseline, suggesting that mechanisms other than removal of the chemoreceptor stimuli may be responsible for the sympathoinhibition (75). However, this was observed in subjects breathing room air post-apnea; Sa_{O_2} was gradually returning to normal. Thus, it cannot be ruled out that increasing Sa_{O_2}, though still below baseline, was partly contributing to the sympathoinhibition. Additionally, we did not assess changes in arterial pCO_2. Thus, more rigorous analysis of the role of chemoreflex unloading in the post-apneic sympathoinhibition is necessary.

Second, activation of the lung inflation reflex could also be contributing to the post-apneic sympathoinhibition. Tidal volume and inspiratory rate are increased immediately post-apnea, both of which increase the sympathoinhibitory effect of lung inflation (51, 52, 63, 68). However, during increased tidal volume breathing the increased sympathoinhibition during the high lung volume phase of respiration is balanced by increased sympathoexcitation during the low lung volume phase of respiration, such that overall MSNA does not change (51, 52, 63, 68). Thus, if lung inflation was the primary mediator of the post-apneic sympathoinhibition, one would expect to see MSNA bursts return during the low lung volume phase of respiration. However, we previously observed that the sympathoinhibition often lasts over several respiratory cycles (75). Thus, it appears that lung inflation is not solely responsible for post-apneic sympathoinhibition. Analysis of MSNA during the low lung volume phase of post-apneic respiration would help to determine the contribution of lung inflation to

post-apneic sympathoinhibition; persistence of sympathoinhibition during the low lung volume phase of post-apneic respiration would indicate that another mechanism was importantly contributing to post-apneic sympathoinhibiton.

Third, arterial baroreflexes may be importantly contributing to post-apneic sympathoinhibition, as arterial pressure remains elevated and often peaks post-apnea (24, 35, 47, 62, 70, 75). As mentioned above, arterial baroreflex control of MSNA appears to be overridden during apnea; however, it is possible that the balance of inputs to the sympathoregulatory centers of the medulla are altered upon apnea termination, such that sympathoinhibitory inputs from the arterial baroreceptors now predominate. The pattern of MSNA and arterial pressure during and following apnea suggest that the role arterial baroreceptors play in the dynamics of MSNA control differs profoundly between the conditions of apnea and apnea termination.

Specific Aims

Research concerning apnea in humans has focused on the control of MSNA during apnea; limited data is available regarding the strong and immediate sympathoinhibition upon the termination of apnea. Therefore, the purpose of the present studies is to determine the mechanism(s) of post-apneic sympathoinhibition in humans. Specifically, these studies will examine the contribution to post-apneic sympathoinhibition of: i) chemoreflex unloading, ii) the lung inflation reflex, and iii)

alterations in carotid baroreflex function. To address these specific aims, the following hypotheses were developed:

I. The normalization of blood gases (i.e., chemoreflex unloading) is not the primary mediator of post-apneic sympathoinhibition.

II. The lung inflation reflex is not the primary mediator of post-apneic sympathoinhibition.

III. Carotid baroreflex control of MSNA diminishes during apnea and returns upon apnea termination.

Experimental Design

The three specific aims were addressed with two separate studies. Details of the protocol and methods of the two studies are presented in chapters 2 and 3; however, a brief summary follows.

Role of Chemoreflex in Post-Apneic Sympathoinhibition

To determine the contribution of chemoreflex unloading to post-apneic sympathoinhibition, subjects performed 15 s end-expiratory apneas followed by breathing of either room air or a gas mixture designed to maintain their end-apneic alveolar gas

percents. Thus, during post-apneic room air-breathing normalization of blood gases occurred, while during post-apneic gas-breathing the chemoreflexes remained stimulated to a degree very similar to end-apnea. Any difference seen in post-apneic sympathoinhibition between the two conditions represents the contribution of chemoreflex unloading to post-apneic sympathoinhibition.

Role of Lung Inflation Reflex in Post-Apneic Sympathoinhibition

To determine the contribution of lung inflation to post-apneic sympathoinhibition, post-apneic MSNA was analyzed as both total MSNA and MSNA occurring only during the low lung volume phase of respiration. If lung inflation is the primary mediator of post-apneic sympathoinhibition, then MSNA bursts would be expected to return during the low lung volume phase of respiration, when the sympathoinhibitory effect of lung inflation is removed. Additionally, MSNA associated with the first two heartbeats post-apnea was quantified, not only to determine the immediacy of the sympathoinhibition but also to compare MSNA during the first post-apneic lung inflation with MSNA during the low lung volume phase of post-apneic respiration.

Role of Carotid Baroreflex in Post-Apneic Sympathoinhibition

To determine whether carotid baroreflex control of MSNA decreased with apnea and returned upon apnea termination, 5 s pulses of –60 Torr NS were delivered during baseline breathing and during the beginning, middle, and end of 20 s end-expiratory apneas, and +30 Torr NP pulses were delivered during baseline and throughout one

minute of post-apneic recovery. The reason only a hypertensive stimulus was utilized during apnea is that MSNA is so elevated during apnea that further increases in response to a hypotensive stimulus would be difficult to observe. Likewise, only a hypotensive stimulus was utilized during post-apneic recovery because MSNA is strongly inhibited post-apnea and further decreases in response to a hypertensive stimulus would be difficult to measure; sympathoinhibition cannot be more than complete. MSNA responses to NS/NP were calculated as the difference between MSNA during the neck pulse and MSNA during a time-matched control in which no neck pulse was delivered. The reason for using time-matched control files rather than using MSNA just prior to NS/NP delivery is because physiological variables are constantly changing during apnea: Sa_{O_2} is falling, arterial pressure is rising, and MSNA is rising. Thus, in these circumstances, a time-matched control file is more appropriate.

Methods

Detailed descriptions of the methods used in the two studies are presented in chapters 2 and 3; however, the complexity of the microneurographic and NS/NP techniques warrant further elaboration.

Microneurography

The technique of microneurography was developed by Vallbo *et al.* and allowed the first direct recordings of impulses from efferent postganglionic sympathetic nerves

serving the vasculature of skeletal muscle (66, 67). The peroneal nerve at the popliteal fossa (postero-lateral aspect of the knee) or the fibular head are commonly used sites for the recording, due to the relatively superficial course of the nerve at these locations. Microneurography in these studies utilized the popliteal fossa site. The leg of the supine subject was comfortably supported, with the knee elevated and slightly bent. Electrical stimulation through the skin identified the location and course of the peroneal nerve, as involuntary twitches are observed in the foot and toes when the nerve is stimulated. Then, a reference electrode was inserted: the sterile tungsten microelectrode (tip diameter 5-10 μm, 35 mm long, Frederick Haer and Co., Bowdoinham, ME, USA) was inserted into the skin 2-3 cm from the identified nerve path and directed towards, but not into, the nerve. A similar recording microelectrode was then inserted into the nerve. Microelectrodes were inserted without local anesthesia since they are so small they do not cause appreciable pain when inserted, and because anesthesia might affect local nerve function. The nerve was slowly and gently probed with the microelectrode while the signal was monitored for insertion discharges and action potentials. A signal processing system rectified and integrated the nerve signal, and amplified it approximately 9×10^4 times (University of Iowa Bioengineering, Iowa City, Iowa, USA). Reproducible activation of pulse-synchronous bursts during an apnea at residual lung volume, and lack of response to skin stroking or startle stimuli, confirmed recording of muscle, and not skin, sympathetic nerve activity. MSNA is expressed as total activity, obtained by summing the areas of the MSNA bursts, and expressed in arbitrary units.

Neck Suction/Neck Pressure

Carotid baroreceptor activation was adjusted via the delivery of suction and pressure to an airtight chamber created by fitting subjects with a cushioned, malleable lead collar which enclosed the anterior two-thirds of the neck. This chamber encloses the carotid sinuses of most individuals; however, Querry *et al.* reported wide variation in carotid sinus location among individuals (45). Thus, appropriate carotid baroreflex responses to NS/NP were confirmed in each subject prior to the start of the study. Pressure of +30 Torr and suction of –60 Torr were generated by a manually controlled, variable pressure source and delivered through large bore two-way solenoid valves (Asco, Florham Park, NJ) to the neck chamber. A pressure transducer (Validyne Engineering Corporation, Northridge, CA) measured neck chamber pressure. The high pressure produced in the chamber during application of +30 Torr NP often created leaks between the neck collar and the subject's neck; thus, it was difficult to maintain steady NP. To correct this, an investigator manually held the collar during all NP pulses such that a tight seal and constant neck chamber pressure were maintained.

The responses to NS/NP pulses are affected by the point in the cardiac and respiratory cycles in which they are delivered (11-15, 36, 64); thus, the timing of NS/NP pulses in relation to these two cycles was kept constant throughout the study. The timing of each NS/NP pulse in relation to the cardiac cycle was controlled by a computer utilizing a customized software package such that each pulse was initiated precisely 50 ms after the R-wave of the ECG and was maintained for 5 s. The timing of each NS/NP pulse in relation to the respiratory cycle was controlled manually by an investigator who

observed the respiratory signal; when subjects reached approximately the last fourth of expiration, the computer-controlled neck pulse was activated. Due to human error and variations in subjects' heart rates and respiratory rates, not all neck pulses were initiated at the correct time in the respiratory cycle. To increase consistency among neck pulse conditions, subjects kept their respiratory rate constant, guided by a computer-generated tone, throughout the protocol. Subjects were asked to maintain a constant tidal volume during baseline breathing, but were allowed to increase tidal volume as necessary post-apnea. Only those neck pulses actually initiated during end-expiration (i.e., the nadir in the respiratory signal) were included in the data analyses.

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CHAPTER II

MECHANISMS OF THE SYMPATHOINHIBITION ASSOCIATED WITH THE TERMINATION OF VOLUNTARY APNEA

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Journal of Applied Physiology

ABSTRACT

Muscle sympathetic nerve activity (MSNA) increases greatly during apnea, and apnea termination is accompanied by a profound and immediate sympathoinhibition, potentially due to the normalization of blood gases, activation of the lung inflation reflex, and arterial baroreflex stimulation. To test the role of chemoreflex unloading in post-apneic sympathoinhibition, we measured MSNA during post-apneic administration of either room air or a hypoxic/hypercapnic gas mixture in ten healthy human subjects. Additionally, we assessed the contribution of lung inflation by isolating post-apneic MSNA associated only with the low lung volume phase of respiration, when the sympathoinhibitory effect of lung inflation is greatly reduced. Post-apneic sympathoinhibition occurred regardless of chemoreflex unloading (room air, apnea vs. post-apnea: 2255±765 vs. 94±51 units/heartbeat, $P = 0.02$; gas mixture, apnea vs. post-apnea: 2329±787 vs. 129±59 units/heartbeat, $P = 0.02$). In addition, post-apneic sympathoinhibition, relative to apnea, persisted during the low lung volume phase of post-apneic respiration. The results indicate that neither chemoreflex unloading nor lung inflation are the primary mediators of post-apneic sympathoinhibition relative to end-apnea.

INDEX TERMS

sympathetic nerve activity, chemoreflex, lung inflation reflex

INTRODUCTION

Sleep apnea is a type of sleep-disordered breathing in which the patient repeatedly ceases to breathe, either due to obstruction of the airway (obstructive sleep apnea) resulting from relaxation of the pharyngeal dilating musculature brought on by sleep (20), or due to failure of the central nervous system to initiate the respiratory signal (central sleep apnea). Apneic events lead to profound increases in muscle sympathetic nerve activity (MSNA) and blood pressure (11, 14, 25, 26). This sympathoexcitation and subsequent blood pressure elevation are primarily chemoreflex-mediated (10, 14, 17, 28). In addition, lack of stimulation to the pulmonary stretch afferents, which are inhibitory to MSNA (9, 21, 29, 30, 32, 37), may also contribute to sympathetic activation during apnea.

Apnea termination is accompanied by a profound and immediate sympathoinhibition (10, 11, 14, 31, 36). Considering the important role that chemoreflex stimulation plays in the apnea-induced sympathoexcitation (10, 14, 17, 28), it follows that the post-apneic sympathoinhibition is due, in part, to the normalization of blood gases (2, 16, 31). However, the abruptness and magnitude of the sympathoinhibition suggest that perhaps other mechanisms are responsible. Our laboratory previously reported that the post-apneic sympathoinhibition occurs before Sa_{O_2} levels return to baseline, suggesting that mechanisms other than removal of the chemoreceptor stimulus may also contribute to the sympathoinhibition (36). However, this was observed in subjects breathing room air post-apnea; Sa_{O_2} was gradually returning to normal. Thus, it cannot be ruled out that

increasing Sa_{O_2}, though still below baseline, was partly contributing to the sympathoinhibition. Additionally, we did not assess changes in arterial pCO_2. Thus, more rigorous analysis of the role of chemoreflex unloading in the post-apneic sympathoinhibition is necessary.

Activation of pulmonary stretch afferents and the continued blood pressure elevation post-apnea may also contribute to the post-apneic sympathoinhibition (2, 31). It has been demonstrated that increased tidal volume breathing, as occurs post-apnea, leads to increased inhibition of MSNA during inspiration (21, 22, 32, 33). Rate of inspiration also modulates MSNA: greater rates of inspiration produce more rapid sympathoinhibition (i.e., sympathoinhibition will occur earlier in inspiration and with a smaller increase in lung volume) (21). These findings suggest that the lung inflation reflex could be an important mediator of the abrupt post-apneic sympathoinhibition, as both tidal volume and rate of inspiration are increased post-apnea.

However, other studies have shown that during increased ventilation, the increased sympathoinhibition during the high lung volume phase of respiration is balanced by increased sympathoexcitation during the low lung volume phase of respiration, such that overall MSNA does not change (21, 22, 32, 33). If lung inflation was the primary mediator of the post-apneic sympathoinhibition, one would therefore expect to see MSNA bursts return during the low lung volume phase of respiration. However, we previously observed that the sympathoinhibition often lasts over several respiratory cycles (36). Therefore, the purpose of the present study was twofold: first, to assess the contribution of chemoreflex unloading to the post-apneic sympathoinhibition

by maintaining chemoreflex stimulation post-apnea, and second, to assess the role of the lung inflation reflex in the post-apneic sympathoinhibition. This latter study aim was addressed by determining whether or not the sympathoinhibition persisted when MSNA was limited to bursts occurring only during the low lung volume phase of respiration. If the lung inflation reflex is the primary mediator of the sympathoinhibition, MSNA would be expected to remain elevated during the low lung volume phase of post-apneic respiration.

METHODS

This study was approved by the University of North Texas Health Science Center Institutional Review Board. Ten healthy volunteers (4 females, 6 males, ages 20-49 years, weight: 73.9 ± 3.6 kg, mean ± SE) participated in the study after giving written, informed consent. All subjects completed a medical history/health questionnaire and passed a physical exam. Subjects were not taking any medication routinely (prescription or over-the-counter). Female subjects tested negative for pregnancy and were not studied during or within two days of menses to eliminate potential confounding effects of menses on fluid metabolism, blood volume, and cardiovascular function. Subjects abstained from exercise and alcohol for 24 hours prior to the study start time, as well as caffeine for 12 hours prior.

Measurements

Heart rate was obtained using a standard limb-lead ECG. Arterial blood pressure was measured non-invasively from beat-to-beat photoplethysmographic recordings at the finger (Finapres Blood Pressure monitor 2300, Ohmeda, Inc., Englewood, CO). This method has been validated against direct arterial pressure recordings (12, 19). A respiratory monitoring band was placed around the subject's abdomen (Grass Instruments, West Warwick, RI), allowing investigators to monitor breathing frequency and insure that all apneas were performed at end-expiration. Pulse oximetry at the forehead assessed Sa_{O_2} (Nellcor, Inc., Hayward, CA). Subjects were also fitted with an airtight mouthpiece and Rudolph valve connected to Douglas bags containing appropriate gas mixtures to allow investigators to control the post-apneic inspired gas and whether their airway was open or closed. Percent oxygen and carbon dioxide inspired and expired were measured at the mouthpiece with mass spectrometry (Medical Gas Analyzer MGA-1100, Perkin-Elmer, Pomona, CA).

Leg MSNA was directly measured from the peroneal nerve at the popliteal fossa utilizing standard microneurographic techniques (4, 28). Two sterile tungsten microelectrodes (tip diameter 5-10 μm, 35 mm long, Frederick Haer and Co., Bowdoinham, ME, USA) were inserted; one was inserted into the peroneal nerve for recording of MSNA and the other served as a reference. Microelectrodes were inserted without local anesthesia since they are so small they do not cause appreciable pain when inserted, and because anesthesia might affect local nerve function. A signal processing system rectified and integrated the nerve signal, and amplified it approximately 9×10^4

times (University of Iowa Bioengineering, Iowa City, Iowa, USA). Reproducible activation during an apnea at residual lung volume, and lack of response to skin stroking or startle stimuli, confirmed recording of muscle, and not skin, sympathetic nerve activity.

Protocol

The study protocol consisted of ten repetitions of the following: 1) one min of baseline, during which subjects breathed a 12% O_2/2% CO_2 gas mixture at a controlled respiratory frequency; 2) a 15 s apnea initiated at the end of a normal tidal expiration; 3) one min of recovery, during which subjects breathed either room air or a gas mixture designed to maintain their end-apneic alveolar gas percents (five of the ten repeats for each condition). In the latter case, subjects breathed the gas mixture for 15 s, after which they were returned to room air for the last 45 s of recovery. During the recovery minute, subjects breathed at the same respiratory frequency as during baseline. The purpose of the post-apneic gas-breathing was to maintain stimulation of the chemoreceptors and determine what effect, if any, this had on the post-apneic sympathoinhibition. The purpose of the mild hypoxic/hypercapnic gas mixture during baseline was twofold: to better simulate sleep apnea, in which patients often experience slight hypoxemia and hypercapnia prior to apnea (23, 24), and to maximize the arterial oxygen desaturation induced by the apnea. Apneas were performed at the end of a normal tidal expiration (i.e., functional residual capacity) to best simulate sleep apnea. Sufficient time was given between repeats to allow subjects to remove the mouthpiece (for comfort's sake) and for

all measured variables to return to baseline levels; however, baseline conditions were typically restored during the minute of recovery. The ten repetitions were performed in random order.

Prior to the study, subjects visited the lab for a familiarization session. During this session, investigators determined the individual subjects' resting breathing frequency. A computer sound file was then used to guide and maintain the subject at that frequency during both baseline and post-apneic breathing, throughout the familiarization session as well as during the study protocol. This was important for insuring that, within a given subject, the conditions were as identical as possible among repeats, especially in terms of arterial oxygen desaturation and carbon dioxide accumulation. Subjects were also instructed to maintain a consistent tidal volume throughout baseline, but were allowed to increase tidal volume (but not respiratory frequency) post-apnea.

The familiarization session was also used to determine the composition of the gas mixture required to maintain the individual subjects' end-apneic alveolar gas levels. The process was as follows. First, the subject breathed the 12% O_2/2% CO_2 gas mixture at controlled respiratory frequency for one min of baseline, followed by a 15 s end-expiratory apnea. At apnea termination, prior to inspiring, the subject performed a forced expiration to residual volume. End-apneic alveolar oxygen and carbon dioxide percents were thus obtained. Baseline and apnea were then repeated, but following apnea the subject breathed an 8% O_2/5% CO_2 gas mixture for approximately 15 s, then performed another forced expiration to residual volume on the next expiration. Alveolar oxygen and carbon dioxide percents were compared to those at end-apnea, and if they were different

by >0.5%, the gas mixture was modified accordingly. This process was repeated until a gas mixture was found which maintained the subject's end-expiratory alveolar gas percents within 0.5% of those obtained at end-apnea. Sa_{O_2} was also observed to insure a lack of oxygen recovery during the post-apneic gas-breathing.

Analyses

Effect of chemoreflex unloading on post-apneic sympathoinhibition. To evaluate the effect of blood gas normalization post-apnea, dependent variables (MSNA, arterial blood pressure, heart rate, and Sa_{O_2}) were averaged for the following time segments: 1) the last 30 s of baseline, 2) the entire 15 s of apnea, 3) the last 5 s of apnea, 4) the first 2 heartbeats post-apnea (i.e., immediately post-apnea), and 5) the first 10 s post-apnea. The timing of MSNA bursts was adjusted to account for the nerve conduction delay (7). Similarly, circulatory distance caused the nadir in arterial Sa_{O_2} (measured at the forehead) to occur several seconds after the end of apnea. To correct for this, the time delay between apnea termination and the nadir in Sa_{O_2} was averaged over the 5 repeats which did not involve gas-breathing post-apnea (i.e., repeats in which Sa_{O_2} recovered after apnea termination). This average delay, calculated separately for each individual subject, was then used for all of that subject's Sa_{O_2} data analyses. Subjects' average delays ranged from 5.6 s to 9.7 s (7.4 ± 0.4 s, mean ± SE).

MSNA is commonly reported in terms of burst frequency; however, this method assumes that all MSNA bursts are of equal significance. While this is acceptable in many cases, apnea-induced MSNA bursts are typically much greater in amplitude and duration

than bursts occurring during baseline or recovery. In addition, MSNA bursts tend to occur with each heartbeat at end-apnea; thus, increases in activity can only be quantified if burst amplitude and duration are considered. For these reasons, MSNA was quantified for each time segment as total activity (expressed in arbitrary units), which equals the sum of the areas of all bursts associated with that time segment. MSNA data were analyzed as both activity/heartbeat and activity/s; the results were not different between the two, so MSNA is reported here only as total activity/heartbeat.

For each dependent variable, a two-way repeated-measures analysis of variance was conducted, with the two factors being time segment and post-apnea condition (room air- vs. gas-breathing). If the ANOVA showed main effect and/or interaction significance, *post hoc* paired t-tests were used to determine specific differences. All statistical analyses were performed at a significance level (α) of 0.05. Values are expressed as means ± SE.

Role of lung inflation reflex in post-apneic sympathoinhibition. If lung inflation is the primary mediator of the post-apneic sympathoinhibition, sympathetic activity should remain elevated during the low lung volume phase of post-apneic respiration; if a mechanism other than lung inflation is the primary mediator of the post-apneic sympathoinhibition, then the sympathoinhibition should likely be maintained throughout both high and low lung volume phases of respiration. To assess the role of lung inflation in post-apneic sympathoinhibition, MSNA associated only with the low lung volume phase of respiration was quantified for five baseline respiratory cycles and the first two post-apneic respiratory cycles. These values were then compared with MSNA during the

last 5 s of apnea, which is held at low lung volume. The low lung volume phase of respiration was defined as the last half of expiration and the first half of inspiration. As described above, MSNA was corrected for the physiological delay such that only bursts resulting from diastoles occurring during low lung volume were included. Again, MSNA was reported as total activity/heartbeat.

To determine if the post-apneic sympathoinhibition was maintained during the low lung volume phase of respiration, and to determine the interaction, if any, of chemoreflex deactivation, a two-way repeated-measures ANOVA was conducted. If the ANOVA showed significance, *post hoc* paired t-tests revealed where, specifically, those differences existed. All statistical analyses were performed at a significance level (α) of 0.05. Values are expressed as means ± SE.

RESULTS

Respiration frequencies used pre- and post-apnea ranged from 10 to 17 breaths/min (13.5 ± 0.8 breaths/min, mean ± SE). The mean gas percentages required to maintain the subjects' end-apneic alveolar gas percents were as follows: O_2: 8.1 ± 0.3% (range: 7.0-9.7%); CO_2: 5.2 ± 0.2% (range: 4.3-6.6%). One subject was unable to hold her breath for the full 15 s; thus, the duration of her apneas was set at 10 s. Her low apnea tolerance was discovered during the familiarization session, so that the post-apnea gas mixture designed to maintain her end-apneic alveolar gas percents was appropriate for the 10 s apneas.

Figures 1 and 2 are raw data tracings from two different subjects; Figure 1 depicts an apnea followed by room air-breathing (Sa_{O_2} recovers), while Figure 2 depicts an apnea followed by gas-breathing (Sa_{O_2} remains low post-apnea). The apnea-induced sympathoexcitation as well as the post-apneic sympathoinhibition are well illustrated in both conditions. The shift in the CO_2 and O_2 tracings during apnea (see Figures 1 and 2, approximately one third into apnea) do not reflect any subject respiratory activity. The reason for the mid-apnea changes in oxygen and carbon dioxide percents is as follows. When subjects initiated an apnea, the tube connecting the mouthpiece to the gas-containing bags remained full of the last gas mixture breathed. To insure that the subjects inspired only the appropriate gas mixture post-apnea, the tube had to be cleared of the pre-apneic gas mixture and filled with the post-apneic gas mixture. Thus, during the apneas, an investigator forced the post-apneic gas mixture into the distal end of the tube, driving the pre-apneic gas mixture out of an open port near the mouthpiece. This caused the shifts in carbon dioxide and oxygen seen during apnea.

Chemoreflex Unloading

Muscle sympathetic nerve activity (MSNA; Figure 3). Total activity/heartbeat was significantly affected by time segment, as expected (ANOVA: $P < 0.001$). MSNA increased dramatically from baseline to the last 5 s of apnea (room air-breathing post-apnea: 159 ± 42 to 2255 ± 765 units/heartbeat, $P = 0.01$; gas-breathing post-apnea: 154 ± 40 to 2329 ± 787 units/heartbeat, $P = 0.01$). Apnea termination reduced MSNA profoundly and immediately (room air-breathing post-apnea: last 5 s of apnea vs. first 2

heartbeats post-apnea, 2255 ± 765 vs. 20 ± 20 units/heartbeat, respectively, $P < 0.01$; gas-breathing post-apnea: last 5 s of apnea vs. first 2 heartbeats post-apnea, 2329 ± 787 vs. 14 ± 10 units/heartbeat, respectively, $P < 0.01$).

Total activity/heartbeat was not significantly affected by post-apneic breathing condition (room air vs. gas; ANOVA: $P = 0.73$) nor was there an interaction between breathing condition and time (ANOVA: $P = 0.92$). Preventing post-apneic chemoreflex unloading by administering a hypoxic/hypercapnic gas mixture did not affect the immediate (i.e., within 2 heartbeats) post-apneic sympathoinhibition (MSNA during first 2 post-apneic heartbeats: room air- vs. gas-breathing, 20 ± 20 vs. 14 ± 10 units/heartbeat, respectively; $P = 0.79$). However, preventing post-apneic chemoreflex unloading did decrease the duration of the post-apneic sympathoinhibition (sympathoinhibition relative to baseline), as MSNA returned to baseline levels within 10 s of apnea termination only when the gas mixture was administered (room air-breathing post-apnea: baseline vs. 10 s post-apnea, 159 ± 42 vs. 94 ± 51 units/heartbeat, respectively, $P = 0.03$; gas-breathing post-apnea: baseline vs. 10 s post-apnea, 154 ± 40 vs. 129 ± 59 units/heartbeat, respectively, $P = 0.60$).

Mean arterial pressure (MAP; Figure 4). MAP was significantly affected by time segment, as expected (ANOVA: $P < 0.001$). MAP increased from baseline to the last 5 s of apnea (room air-breathing post-apnea: 89 ± 3 to 96 ± 4 mmHg, $P < 0.01$; gas-breathing post-apnea: 88 ± 4 to 96 ± 4 mmHg, $P = 0.01$). During the first 2 post-apneic heartbeats, MAP continued to increase slightly but not significantly, and remained elevated throughout the 10 s post-apneic analysis period. MAP was not affected by post-

apneic breathing condition, either immediately post-apnea (within 2 heartbeats) or within 10 s post-apnea (room air vs. gas; ANOVA: $P = 0.78$), nor was there an interaction between breathing condition and time (ANOVA: $P = 0.85$).

Heart rate (Figure 5). Heart rate was significantly affected by time segment, as expected (ANOVA: $P < 0.001$). Heart rate tended to decrease with apnea (room air-breathing post-apnea: baseline vs. last 5 s of apnea, 68 ± 3 vs. 62 ± 3 bpm, respectively, $P = 0.07$; gas-breathing post-apnea: baseline vs. last 5 s of apnea, 67 ± 3 vs. 61 ± 3 bpm, respectively, $P < 0.05$). Heart rate increased from end-apnea levels immediately upon apnea termination (room air-breathing post-apnea: last 5 s of apnea vs. first 2 heartbeats post-apnea, 62 ± 3 vs. 73 ± 3 bpm, respectively, $P = 0.0001$; gas-breathing post-apnea: last 5 s of apnea vs. first 2 heartbeats post-apnea, 61 ± 3 vs. 74 ± 3 bpm, respectively, $P < 0.0001$), and was back to baseline levels within 10 s post-apnea (both $P > 0.80$).

Post-apneic breathing condition did not affect heart rate (ANOVA: $P = 0.94$) nor was there an interaction between breathing condition and time (ANOVA: $P = 0.15$). Although heart rate during the first 2 post-apneic beats was significantly higher than baseline when the subjects were breathing the gas mixture, but not when they were breathing room air (room air-breathing post-apnea: baseline vs. 2 heartbeats post-apnea, 68 ± 3 vs. 73 ± 3 bpm, respectively, $P = 0.059$; gas-breathing post-apnea: baseline vs. 2 heartbeats post-apnea, 67 ± 3 vs. 74 ± 3 bpm, respectively, $P = 0.005$), this difference between post-apneic conditions is not physiologically significant (i.e., one beat·min^{-1}).

Arterial oxygen saturation (Sa_{O_2}; Figure 6) and end-tidal CO_2. As expected, Sa_{O_2} decreased with apnea (time segment ANOVA: $P < 0.001$), increased towards

baseline levels during the room air-breathing recovery, and remained low during the gas-breathing recovery. Post-apneic condition significantly affected Sa_{O_2} levels, as was the goal of the protocol (room air vs. gas; ANOVA: $P < 0.001$) and there was a significant interaction between post-apneic breathing condition and time (ANOVA: $P < 0.001$). Sa_{O_2} was significantly lower during gas-breathing recovery than during room air-breathing recovery at both post-apneic time points (2 heartbeats post-apnea: room air- vs. gas-breathing, 86 ± 2 vs. 85 ± 1 %, respectively, $P = 0.001$; 10 s post-apnea: room air- vs. gas-breathing, 91 ± 1 vs. 86 ± 1 %, respectively, $P < 0.0001$). Sa_{O_2} decreased significantly from the last 5 s of apnea to the first 2 heartbeats post-apnea (both conditions $P < 0.001$); however, this was not due to a continued decrease of Sa_{O_2} after apnea termination, but rather was the result of the analyses in that the duration of the two time segments was different (i.e., 5 s during which Sa_{O_2} was falling to its nadir vs. approximately 1.6 s or 2 heartbeats during which Sa_{O_2} was either just beginning to recover or remaining at its nadir, depending on the post-apneic condition). During the post-apneic gas-breathing condition, there was no significant difference in Sa_{O_2} from 2 heartbeats post-apnea to 10 s post-apnea, confirming that the gas mixtures used were successful in preventing Sa_{O_2} recovery (2 heartbeats post-apnea vs. 10 s post-apnea, 85 ± 1 vs. 86 ± 1 %, respectively, $P = 0.35$).

End-tidal CO_2 was significantly greater during the post-apneic gas-breathing condition than the post-apneic room air-breathing condition, as designed (room air- vs. gas-breathing, 5.4 ± 0.18 vs. 6.4 ± 0.18 %, respectively, $P < 0.0001$). End-tidal CO_2 was 5.9 ± 0.19 % during baseline, for apneas of both conditions ($P = 0.90$).

Lung Inflation Reflex

MSNA during low lung volume only (Figure 7). Total activity/heartbeat was significantly affected by time segment (ANOVA: $P = 0.007$), and followed a pattern very similar to that shown in Figure 3. This resulted from the fact that virtually all sympathetic bursts occurring during the baseline and post-apnea time segments occurred during the low lung volume phase of the respiratory cycle; thus, low lung volume MSNA reflected total MSNA. Apnea significantly increased MSNA (room air-breathing post-apnea: baseline vs. last 5 s of apnea, 294 ± 87 vs. 2255 ± 765 units/heartbeat, respectively, $P = 0.01$; gas-breathing post-apnea: baseline vs. last 5 s of apnea, 277 ± 77 vs. 2329 ± 787 units/heartbeat, respectively, $P = 0.01$). Within the first two respiratory cycles post-apnea, low lung volume MSNA had returned to baseline levels during gas-breathing, but remained below pre-apnea baseline during room air-breathing.

There was no significant effect of post-apneic breathing condition on low lung volume MSNA (ANOVA: $P = 0.25$) nor was there an interaction between breathing condition and time (ANOVA: $P = 0.55$). However, similar to the 10 s post-apnea time segment in Figure 3, paired t-tests determined low lung volume MSNA to be significantly less during post-apneic breaths than during baseline breaths *only* when room air was administered post-apnea (room air-breathing post-apnea: baseline vs. post-apnea, 294 ± 87 vs. 182 ± 88 units/heartbeat, respectively, $P = 0.04$; gas-breathing post-apnea: baseline vs. post-apnea, 277 ± 77 vs. 324 ± 173 units/heartbeat, respectively, $P = 0.72$).

DISCUSSION

The decrease in MSNA to levels significantly below baseline within the first 2 heartbeats post-apnea, regardless of post-apneic breathing condition, demonstrates the abruptness and near completeness of the sympathoinhibition associated with apnea termination. Low lung volume MSNA during post-apneic gas-breathing (i.e., post-apneic MSNA when the sympathoinhibitory effects of chemoreflex unloading and lung inflation have been removed; far right column of Figure 7) was not different from baseline. These data demonstrate that neither chemoreflex unloading nor the lung inflation reflex is the primary mediator of the profound sympathoinhibition observed upon apnea termination. While both reflexes appear to contribute to MSNA levels post-apnea and may perhaps be involved in the sympathoinhibition relative to baseline, another mechanism(s) seems to be responsible for the sympathoinhibition relative to end-apnea.

Role of chemoreflex in post-apneic sympathoinhibition

The first aim of this study was to determine whether withdrawal of chemoreflex stimuli upon apnea termination contributes primarily to post-apneic sympathoinhibition. If it does, then maintenance of the chemoreflex stimuli by breathing a gas which maintains a similar blood gas state to that occurring during the end of apnea should minimize the post-apneic sympathoinhibition. Apnea increased total MSNA approximately 15-fold from baseline MSNA; however, post-apnea, MSNA had either returned to baseline MSNA or was significantly decreased from baseline MSNA,

regardless of whether the subjects were breathing room air or a gas mixture designed to maintain their end-apnea alveolar gas levels. Thus, chemoreflex unloading does not appear to be importantly involved in the profound sympathoinhibition observed from end-apnea to post-apnea. This is in agreement with previous studies which reported that post-apneic sympathoinhibition occurs before the return of Sa_{O_2} to baseline Sa_{O_2} (10, 36). Chemoreflex stimulation has been shown to contribute importantly to the sympathetic and pressor responses to apnea (10, 14, 17, 28, 34). However, these data indicate that chemoreflex unloading does not appear to play an important role in the post-apneic sympathoinhibition relative to end-apnea. Therefore, other sympathoinhibitory mechanisms which are active at apnea termination must override the sympathoexcitatory input from the chemoreceptors, even when subjects are breathing a severely hypoxic/hypercapnic gas mixture.

While chemoreflex unloading does not appear to play an important role in post-apneic sympathoinhibition relative to end-apnea, it does appear to contribute to the post-apneic sympathoinhibition relative to baseline. This point is illustrated in the "post-apnea" data of Figure 7. These data represent post-apneic MSNA when the inhibitory effect of lung inflation is removed (i.e., during low lung volume). When blood gases were allowed to normalize with breathing of room air, MSNA was significantly below baseline MSNA, even during low lung volume when MSNA is at its highest in the respiratory cycle (5, 6, 22, 32). However, when chemoreflex unloading was prevented with gas-breathing, MSNA was not different from pre-apnea baseline, which raises the possibility of a chemoreflex adaptation during the apnea: the gas mixtures breathed by

subjects post-apnea averaged 8.1 ± 0.3% O_2 and 5.2 ± 0.2% CO_2, a much stronger chemoreflex stimulus than the 12% O_2/2% CO_2 gas mixture breathed during baseline, yet MSNA was not different. Alternatively, and more likely, the stronger chemoreceptor stimulus post-apnea may have been counterbalanced by other sympathoinhibitory mechanisms which were not present during baseline conditions, such as increased arterial baroreceptor activity.

Role of lung inflation reflex in post-apneic sympathoinhibition

The second aim of this study was to determine whether activation of pulmonary stretch afferents (i.e., the lung inflation reflex) at apnea termination contributes importantly to post-apneic sympathoinhibition. The lung inflation reflex inhibits both sympathetic (9, 21, 29, 30, 32, 37) and parasympathetic (1, 6) nerve activity, and data from animal studies have shown that lung inflation can reduce or abolish chemoreflex-induced bradycardia (1, 3, 8). Data from humans also indicate that lung inflation can override excitatory input from the chemoreceptors. Smith and colleagues (27) reported that in spite of an overall increase in MSNA during breathing of a hypoxic/hypercapnic gas mixture, MSNA during the high lung volume phase of respiration was actually decreased from baseline. However, because total MSNA was increased, MSNA during low lung volume must have greatly increased. Thus, while stimulation of the lung inflation reflex may be able to explain the immediate (i.e., within 2 heartbeats, which always occurred during the first inspiration) post-apneic sympathoinhibition in the face of

continued chemoreceptor loading in our subjects, it cannot explain the continued sympathoinhibition relative to end-apnea, which persisted over several respiratory cycles.

The lung inflation reflex is clearly operative post-apnea, as virtually all of the post-apneic MSNA bursts occurred during the low lung volume phase of respiration. For this reason, low lung volume MSNA is representative of total MSNA. Values of MSNA during baseline and post-apnea are greater in Figure 7 than Figure 3 because MSNA is reported as units/heartbeat, and fewer heartbeats were used for the values in Figure 7 (that is, all heartbeats associated with high lung volume were removed). But the overall relationship of baseline to apnea and post-apnea MSNA data remained unchanged, regardless of whether the data were derived as total activity or activity during low lung volume alone. Further evidence that the lung inflation reflex was functioning post-apnea is demonstrated by comparing the "2HB post" data of Figure 3 with the "post-apnea" data of Figure 7; the former represents MSNA during pulmonary stretch receptor loading (the first 2 heartbeats post-apnea always occurred during the first rapid inspiration) while the latter represents MSNA during pulmonary stretch receptor unloading. Lung inflation was associated with significant sympathoinhibition during the room air condition (high lung volume, i.e., first 2 heartbeats post-apnea vs. low lung volume, 20 ± 20 vs. 182 ± 88 units/heartbeat, respectively, $P < 0.05$) and showed a trend for sympathoinhibition during the gas mixture condition (high lung volume, i.e., first 2 heartbeats post-apnea vs. low lung volume, 14 ± 10 vs. 324 ± 173 units/heartbeat, respectively, $P = 0.11$). Although the sympathoinhibition did not reach statistical significance for the gas mixture condition, all but two subjects had increased activity during low lung volume compared with high lung

volume, and the remaining two had no activity during either phase of respiration (i.e., they displayed complete post-apneic sympathoinhibition). As mentioned above, other investigators have also observed the continued operation of the lung inflation reflex concomitant with a chemoreflex challenge (22, 27).

In spite of the lung inflation reflex being operative post-apnea, post-apneic sympathoinhibition relative to end-apnea occurred regardless of whether the pulmonary stretch receptors were loaded (during the first 2 heartbeats post-apnea) or unloaded (during the low lung volume phase of respiration). While lung inflation likely contributed to the decreased MSNA immediately post-apnea compared to MSNA over the first 10 s post-apnea (Figure 3), the fact that MSNA remained at or below baseline even when the pulmonary stretch receptors were unloaded indicates that this reflex is not the primary mediator of the sympathoinhibition relative to end-apnea. In fact, it is possible that lung inflation does not even contribute importantly to the immediate post-apneic sympathoinhibition. Subjects terminating inspiratory-capacity apneas also appear to experience immediate sympathoinhibition, although they terminate their apneas with an expiration rather than an inspiration (see Figures 3 and 4 of reference) (15)).

Other potential mediators of the post-apneic sympathoinhibition

The control of MSNA during apnea and immediately post-apnea is complex and involves both competing and complicit mechanisms of control including chemoreflexes, the lung inflation reflex, baroreflexes, and central respiratory drive (36). These data suggest that neither chemoreceptor unloading nor lung inflation alone is the primary

mediator of post-apneic sympathoinhibition. Central interaction among these reflexes is complex and incompletely understood, but likely plays an important role that is beyond the scope of these data. Arterial blood pressure increases throughout the apnea and remains elevated post-apnea as a result of the strong sympathoexcitation during apnea (13). The increase in arterial pressure observed at end-apnea would be expected to inhibit MSNA via the arterial baroreflex; the fact that profound increases in arterial pressure and MSNA occur simultaneously indicate that arterial baroreceptor sympathoinhibitory input is being overridden, at least in part, by the sympathoexcitatory influences of the chemoreceptors and unloaded pulmonary stretch afferents.

Upon apnea termination, arterial pressure remains elevated (or even peaks) while MSNA is strongly inhibited, even when chemoreflex activation is maintained. Perhaps some change in the balance of inputs to the brainstem at apnea termination allows the arterial baroreceptor sympathoinhibitory input to dominate over the chemoreceptor sympathoexcitatory input. If this were true, one might also expect to see dominant arterial baroreflex control of heart rate post-apnea, but apnea termination is usually accompanied by relative tachycardia rather than bradycardia. However, Wallin and Eckberg (35) have suggested that central processing of arterial baroreceptor input is different for the parasympathetic (i.e., cardiac) and sympathetic branches of the reflex. This postulation was based on their observation of different degrees and time courses of adaptation of these two branches to the same baroreceptor stimulus. Additionally, Narkiewicz *et al.* (18) reported impairment of the sympathetic but not the cardiac branch of the arterial baroreflex in obstructive sleep apnea patients, suggesting a possible

disassociation between the two branches of the reflex. At this time, it is unknown what role arterial baroreceptors play in the dynamics of MSNA control during and following apnea. Nevertheless, the present data suggest that the role of baroreflex input during versus after an apnea is likely different.

Central respiratory drive is suppressed by higher brain centers during voluntary apnea in our subjects, and release from this suppression and return of central respiratory drive upon apnea termination could also have been contributing to the post-apneic sympathoinhibition. The abruptness of the sympathoinhibition supports a central mechanism. Previous studies have altered respiratory motor output utilizing mechanical ventilation and added inspiratory resistance; the former can completely eliminate all active inspiratory efforts and the latter increases respiratory motor output (22, 32). Yet, in spite of the wide variations in respiratory motor output produced under these conditions, the respiratory pattern of MSNA was unchanged (22, 32) and greatly increased respiratory motor output did not decrease total MSNA (32). While this does not exclude the possibility of critical involvement of central respiratory drive in post-apneic sympathoinhibition, it suggests that perhaps other mechanisms may be more important.

Input from higher brain centers to suppress respiration during voluntary apnea and the subsequent release from this suppression upon apnea termination may have directly affected reflex control of MSNA, separate from its effect on central respiratory drive. Anxiety associated with breath-holding may have partly contributed to the sympathoexcitation observed at end-apnea, in spite of the fact that subjects had been

familiarized with the protocol. Thus, our subjects may have experienced a conscious "relief" upon apnea termination which could have contributed to the post-apneic sympathoinhibition. However, studies measuring MSNA during spontaneous apneas during sleep have reported similar post-apneic sympathoinhibition (11, 14, 31), and it is doubtful that these sleeping patients experienced higher brain center "relief" upon resumption of respiration, as sleep apnea patients are typically unaware of the apneas. Therefore, this mechanism of post-apneic sympathoinhibition is unlikely.

Study limitations

One of the limitations of this study involves the lung inflation analysis. To determine the contribution of the lung inflation reflex to the post-apneic sympathoinhibition, MSNA associated only with low lung volume during baseline and post-apneic respiration was compared to MSNA at end-apnea, when lung volume was held at functional residual capacity. Therefore, measurements during baseline and post-apnea reflect MSNA during dynamic low lung volume (at or near functional residual capacity), while measurements at end-apnea reflect MSNA during static low lung volume. Sympathetic responses to lung inflation have been shown to differ depending on whether the inflation is dynamic or static (15), but such a differentiation is unknown between static and dynamic *lack* of pulmonary afferent stimulation; however, we predict that it would be less important since the inhibitory influence of inflation is removed in either case. In addition, the fact that low lung volume MSNA during baseline and post-apneic gas-breathing were not different provides evidence that some other mechanism

besides lung inflation is inhibiting MSNA post-apnea. Studies have shown that chemoreflex stimulation increases tidal volume and total MSNA without changing the respiratory modulation of MSNA (i.e., sympathoinhibition during high lung volume and sympathoexcitation during low lung volume) (22, 27). In other words, the chemoreflex-induced sympathoexcitation is reflected in low lung volume MSNA. Yet, during post-apneic gas-breathing in our subjects when the chemoreflex stimuli were much higher than during baseline, low lung volume MSNA was not different from baseline. This suggests that some other post-apneic mechanism is inhibiting MSNA during low lung volume, when pulmonary afferents are not activated.

Another limitation relates to the ventilatory response after apnea. Post-apnea, subjects were exposed to either room air or a gas mixture which maintained their end-apnea alveolar gas levels, and differences in MSNA between these two conditions were attributed to chemoreflex unloading. However, breathing the gas mixture likely resulted in increased ventilation (via increased tidal volume only, as respiratory frequency was controlled) compared to breathing room air, and this could itself have contributed to any differences observed. Several studies have examined the effect of hyperventilation (both room air-breathing and isocapnic and thus with no concomitant chemoreflex stimulation) on MSNA and found that total MSNA was unchanged (i.e., the increased sympathoinhibition during inspiration was balanced by the increased sympathoexcitation during expiration) (21, 22, 32, 33). Thus, MSNA results for the first 10 s post-apnea should not have been affected by the modestly increased ventilation itself, as they include MSNA over complete respiratory cycles. Likewise, MSNA calculated over the first 2

heartbeats post-apnea should also not have been affected by the gas mixture-induced increased ventilation, as this measurement is from immediately post-apnea while subjects were taking their first inspiration. Additionally, the possibility of increased ventilation during gas-breathing post-apnea does not affect our conclusion that chemoreceptor unloading is not the primary mediator of the sympathoinhibition relative to end-apnea. Increased ventilation could also have affected our results via its effect on arterial baroreflex function. Isocapnic hyperventilation has been shown to decrease arterial baroreflex control of MSNA (33). If this had occurred during post-apnea in our subjects, it would have increased our values of post-apneic MSNA, as arterial pressure was elevated post-apnea and the baroreflexes would have been less able to inhibit MSNA in response. Because we observed post-apneic sympathoinhibition in our subjects, this effect of increased ventilation is not confounding our results.

In conclusion, chemoreflex unloading following apnea termination did not contribute importantly to the post-apneic sympathoinhibition relative to end-apnea; however, it did appear to play a role in the post-apneic sympathoinhibition relative to baseline, as this only occurred when blood gases were allowed to normalize. The lung inflation reflex was operative post-apnea, but while it may have been responsible for the immediate post-apneic sympathoinhibition relative to baseline, it would appear not to be the primary mediator of the sustained sympathoinhibition relative to end-apnea.

ACKNOWLEDGEMENTS

The authors thank the subjects for their cooperation and contribution to this work. This research was supported by NIH grant HL-49266, for which we are grateful.

This research was submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy for Nicolette Muenter Swift.

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FIGURE LEGENDS

Figure 1. Raw data tracing depicting the last part of baseline (12% O_2/2% CO_2 gas-breathing), apnea, and the first part of room air-breathing recovery. Note that neither the MSNA nor the Sa_{O_2} tracings have yet been corrected for their respective time delays. BP = arterial pressure in mmHg; Insp Flow = inspiratory airflow in liters; CO_2 and O_2 = percent in inspired and expired air; Sa_{O_2} = percent arterial oxygen saturation.

Figure 2. Raw data tracing depicting the last part of baseline (12% O_2/2% CO_2 gas-breathing), apnea, and the first part of recovery in which the subject first breathed a gas mixture designed to maintain the subject's end-apnea blood gas levels, followed by room air-breathing. Note that neither the MSNA nor the Sa_{O_2} tracings have yet been corrected for their respective time delays. BP = arterial pressure in mmHg; Insp Flow = inspiratory airflow in liters; CO_2 and O_2 = percent in inspired and expired air; Sa_{O_2} = percent arterial oxygen saturation.

Figure 3. Effect of post-apneic chemoreflex unloading on MSNA. End-expiratory apnea increased MSNA approximately 15-fold from baseline, and apnea termination was associated with an immediate and profound sympathoinhibition. 2HB post = the first 2 heartbeats post-apnea. *Significantly different from baseline, $P<0.05$.

Figure 4. Effect of post-apneic chemoreflex unloading on mean arterial pressure (MAP). MAP increased significantly above baseline by the last 5 s of apnea, increased slightly but not significantly more during the first 2 heartbeats post-apnea (2HB post), and remained elevated over the 10 s post-apnea. *Significantly greater than baseline, $P<0.05$.

Figure 5. Effect of post-apneic chemoreflex unloading on heart rate. Moderate bradycardia accompanied the last 5 s of apnea, and apnea termination was accompanied by tachycardia. At both time segments, the change in heart rate was significant for the post-apneic gas-breathing condition but not the room air-breathing condition; however, this difference is not functionally significant (difference in average values = 1 bpm for both time segments). Heart rate was back to baseline within 10 s post-apnea for both conditions. 2HB post = the first 2 heartbeats post-apnea. *Significantly different than baseline, $P<0.05$. †Significantly greater than apnea-last 5s, $P<0.001$.

Figure 6. Effect of post-apneic chemoreflex unloading on arterial oxygen saturation (Sa_{O_2}). End-expiratory apnea significantly decreased Sa_{O_2} below baseline (subjects are breathing a 12% O_2/2% CO_2 gas mixture during baseline). Sa_{O_2} partially recovered within 10 s post-apnea during the room air-breathing condition. As intended, Sa_{O_2} did not recover during gas-breathing post-apnea and was significantly below room air-breathing values at both post-apneic time segments (for explanation of why Sa_{O_2} is lower immediately post-apnea than during the last 5 s of apnea, see text). 2HB post = the first 2

heartbeats post-apnea. *Significantly less than baseline, $P<0.001$. †Significantly less than room air, $P<0.01$.

Figure 7. MSNA responses to apnea and apnea termination with the sympathoinhibitory effect of lung inflation removed (i.e., MSNA associated only with the low lung volume phase of respiration). The significant post-apneic decrease in MSNA compared to baseline during only the room air-breathing condition reflects the contribution of chemoreceptor unloading to the post-apneic sympathoinhibition relative to baseline. *Significantly different from baseline, $P<0.05$.

Figure 1

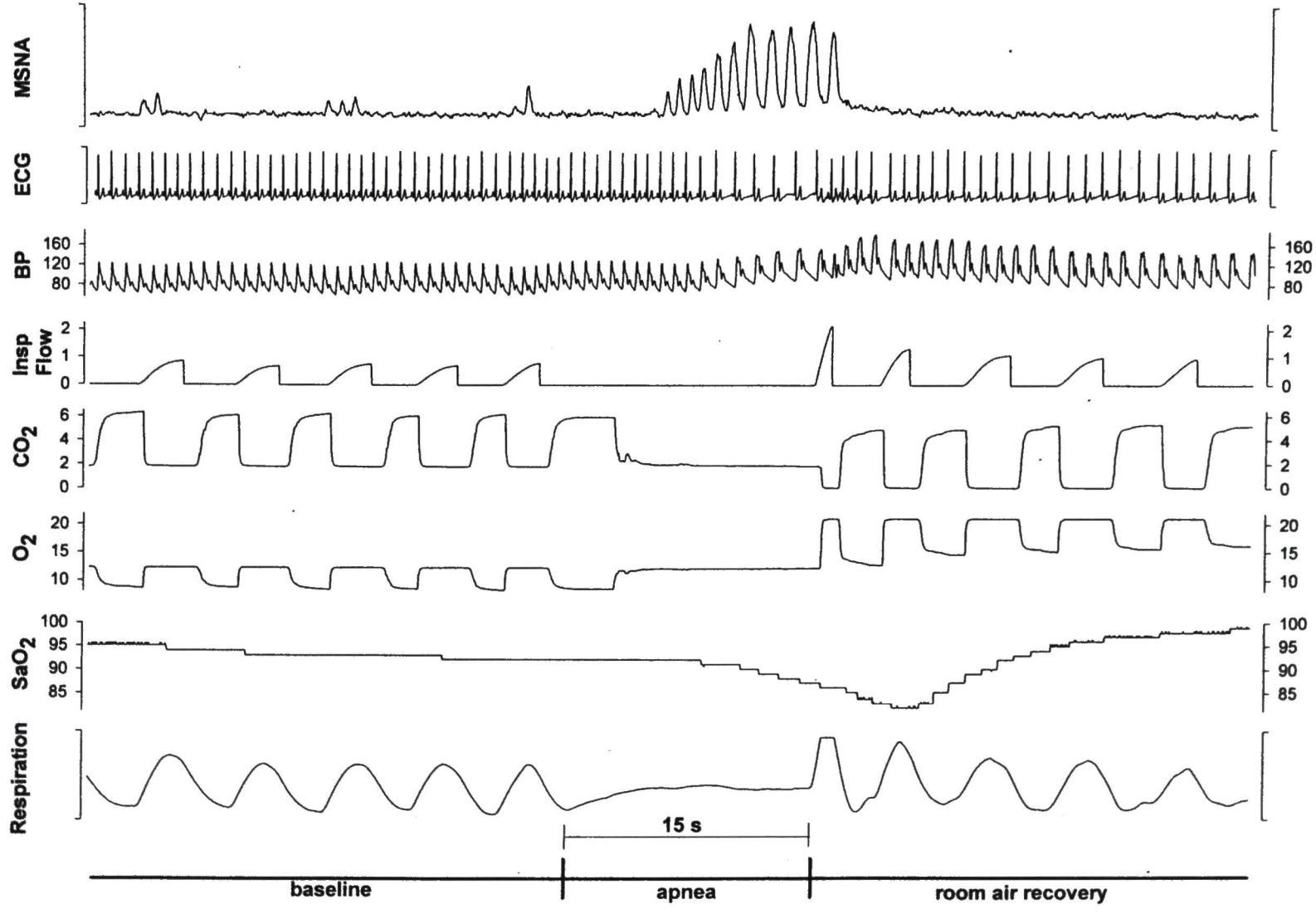

MSNA
ECG
BP
160
120
80
Insp Flow
2
1
0
CO_2
6
4
2
0
O_2
20
15
10
SaO_2
100
95
90
85
Respiration
15 s
baseline
apnea
room air recovery

Figure 2

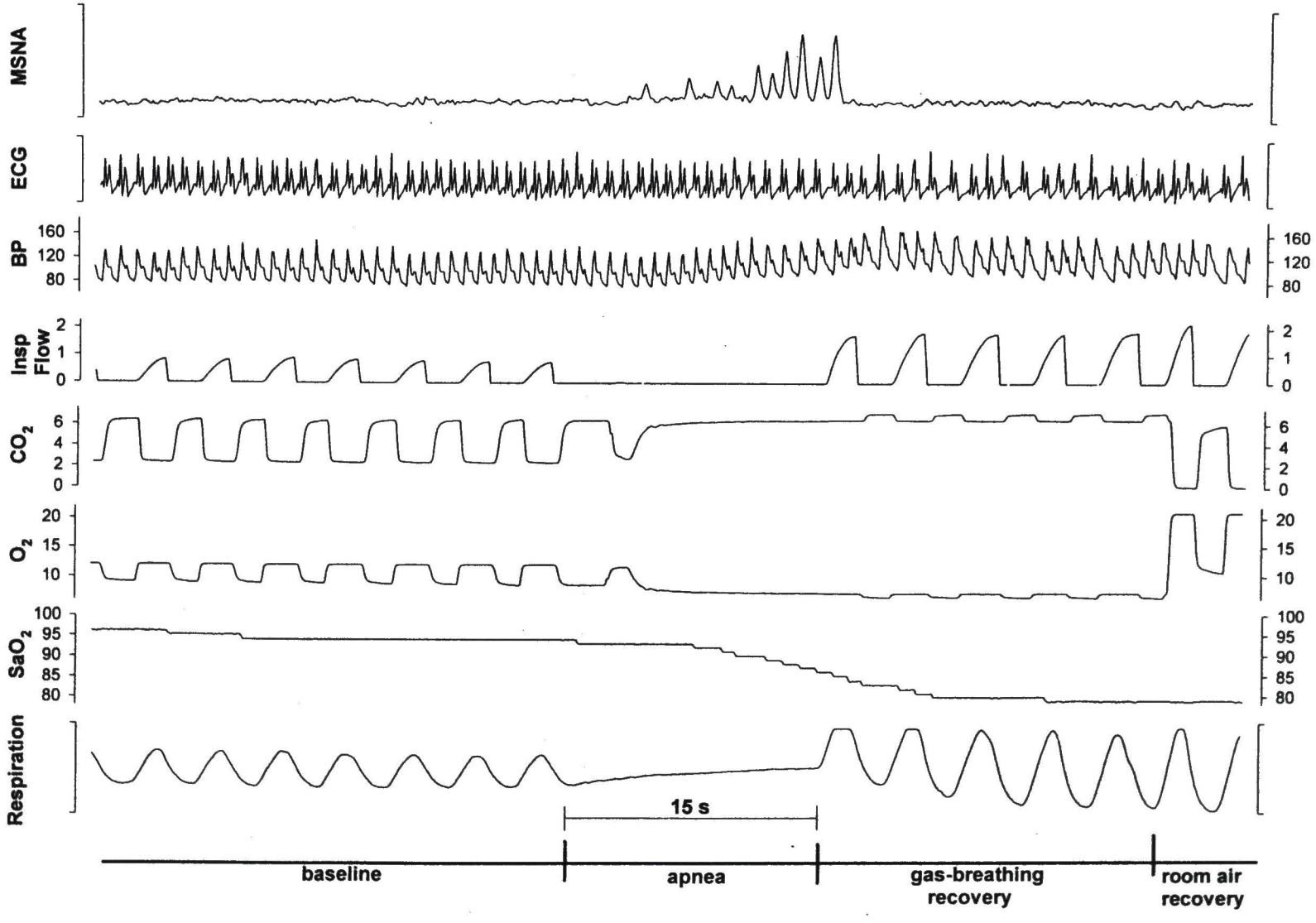

MSNA
ECG
BP
160
120
80
Insp Flow
2
1
0
CO_2
6
4
2
0
O_2
20
15
10
SaO_2
100
95
90
85
80
Respiration
15 s
baseline
apnea
gas-breathing recovery
room air recovery

Figure 3

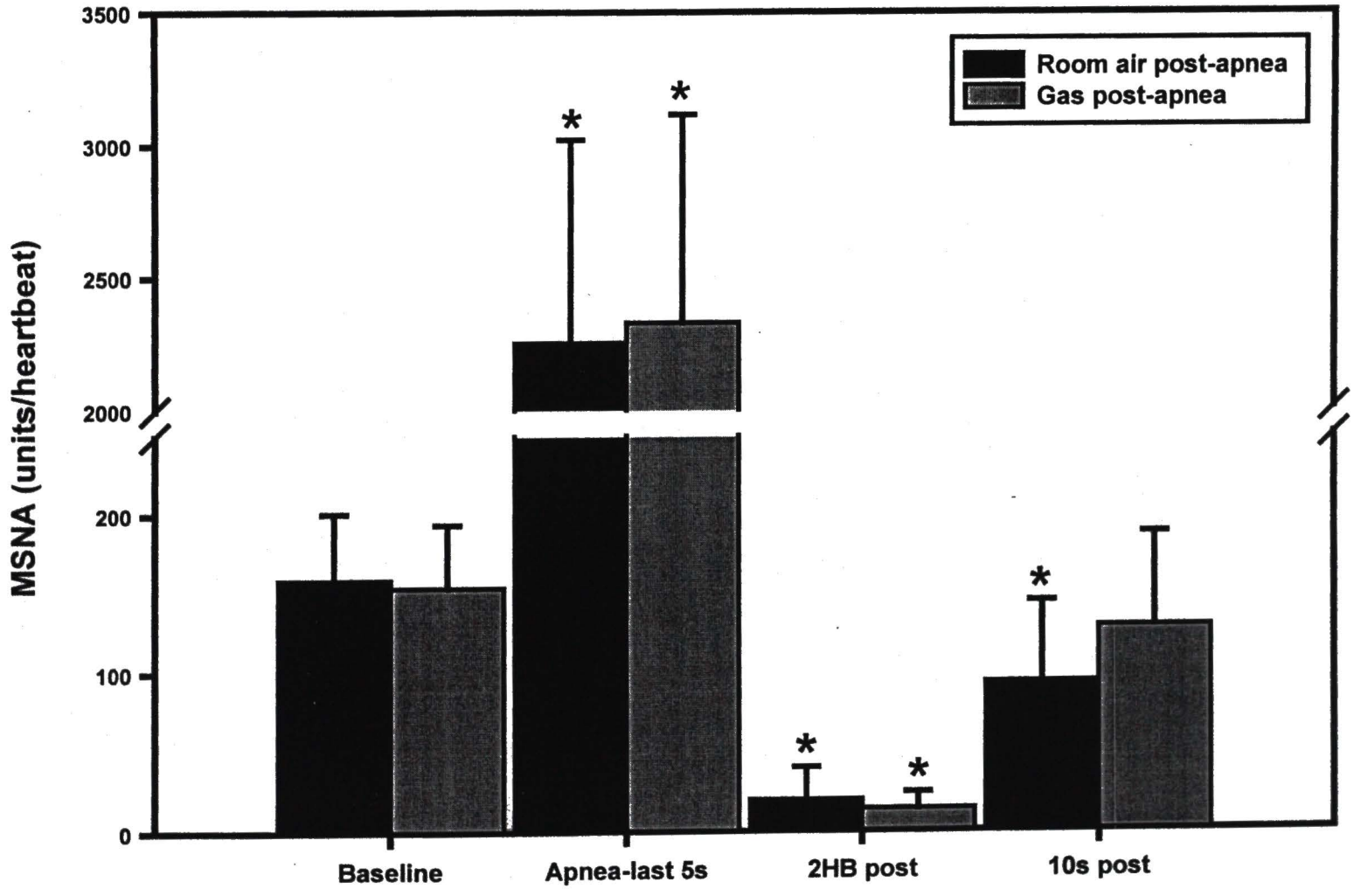

MSNA (units/heartbeat)
3500
3000
2500
2000
200
100
0
Room air post-apnea
Gas post-apnea
*
*
*
*
*
Baseline
Apnea-last 5s
2HB post
10s post

Figure 4

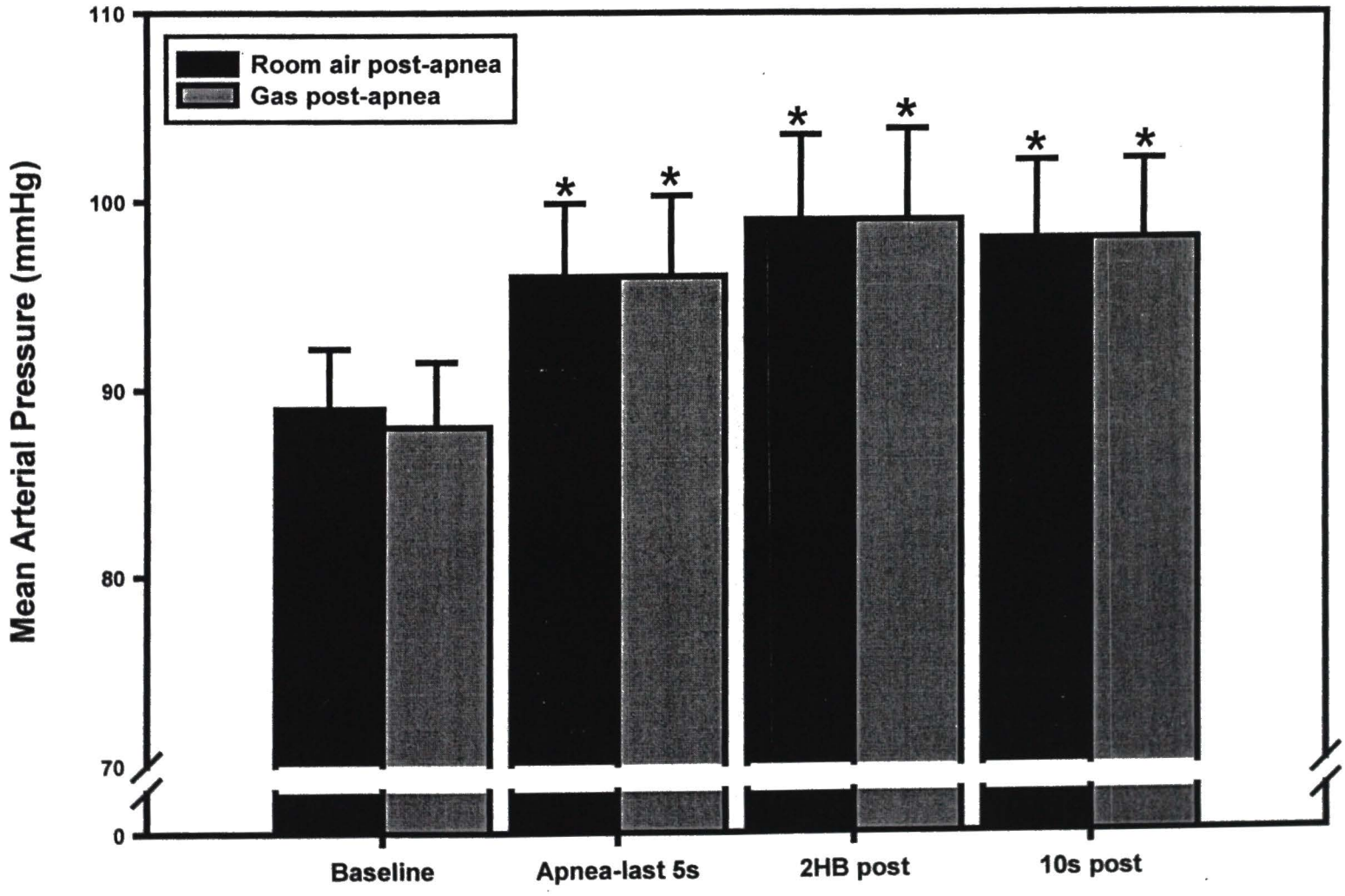

Mean Arterial Pressure (mmHg)
110
100
90
80
70
0
Room air post-apnea
Gas post-apnea
*
*
*
*
*
*
Baseline
Apnea-last 5s
2HB post
10s post

Figure 5

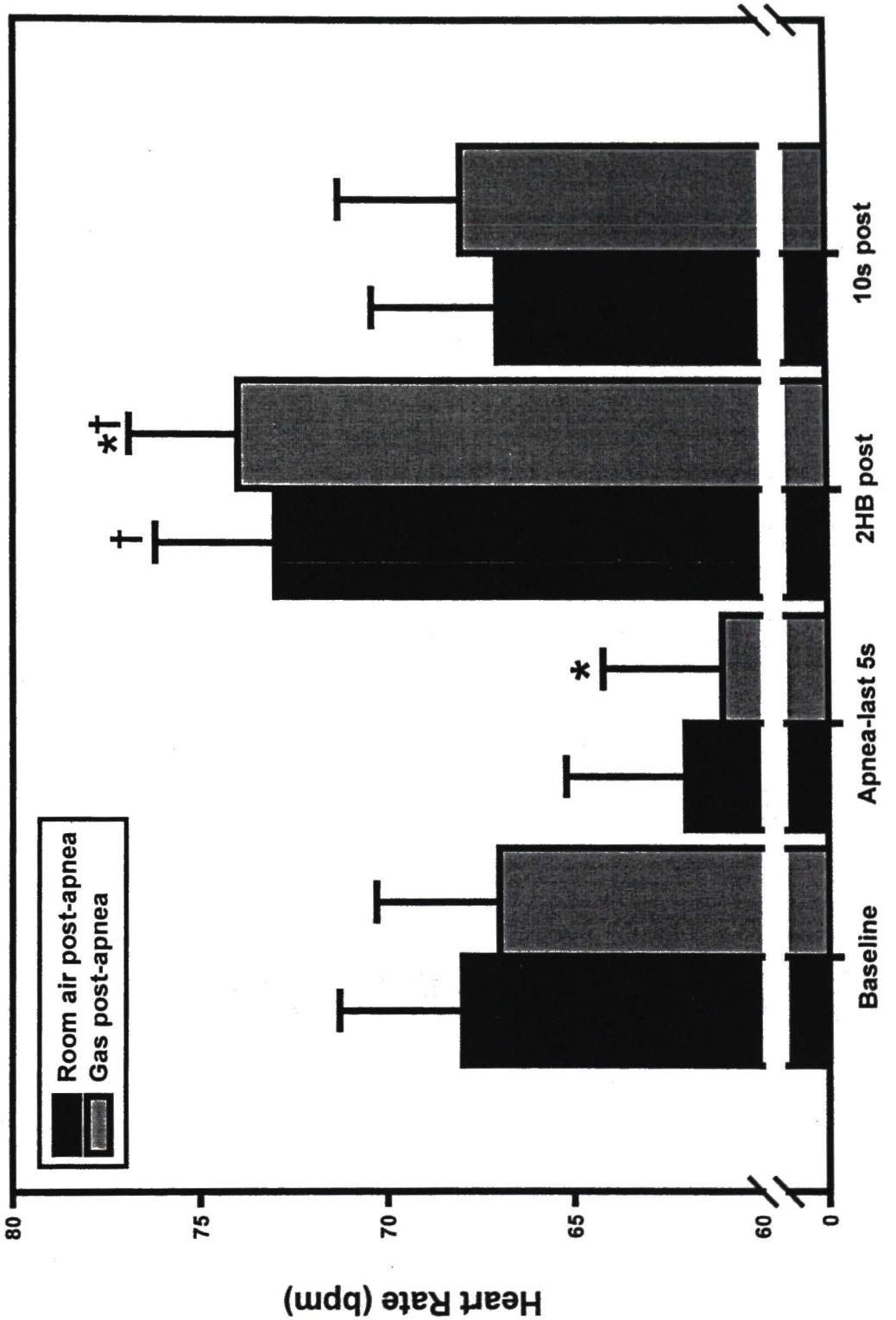

Heart Rate (bpm)
80
75
70
65
60
0
Room air post-apnea
Gas post-apnea
Baseline
Apnea-last 5s
2HB post
10s post
*
†
*†

Figure 6

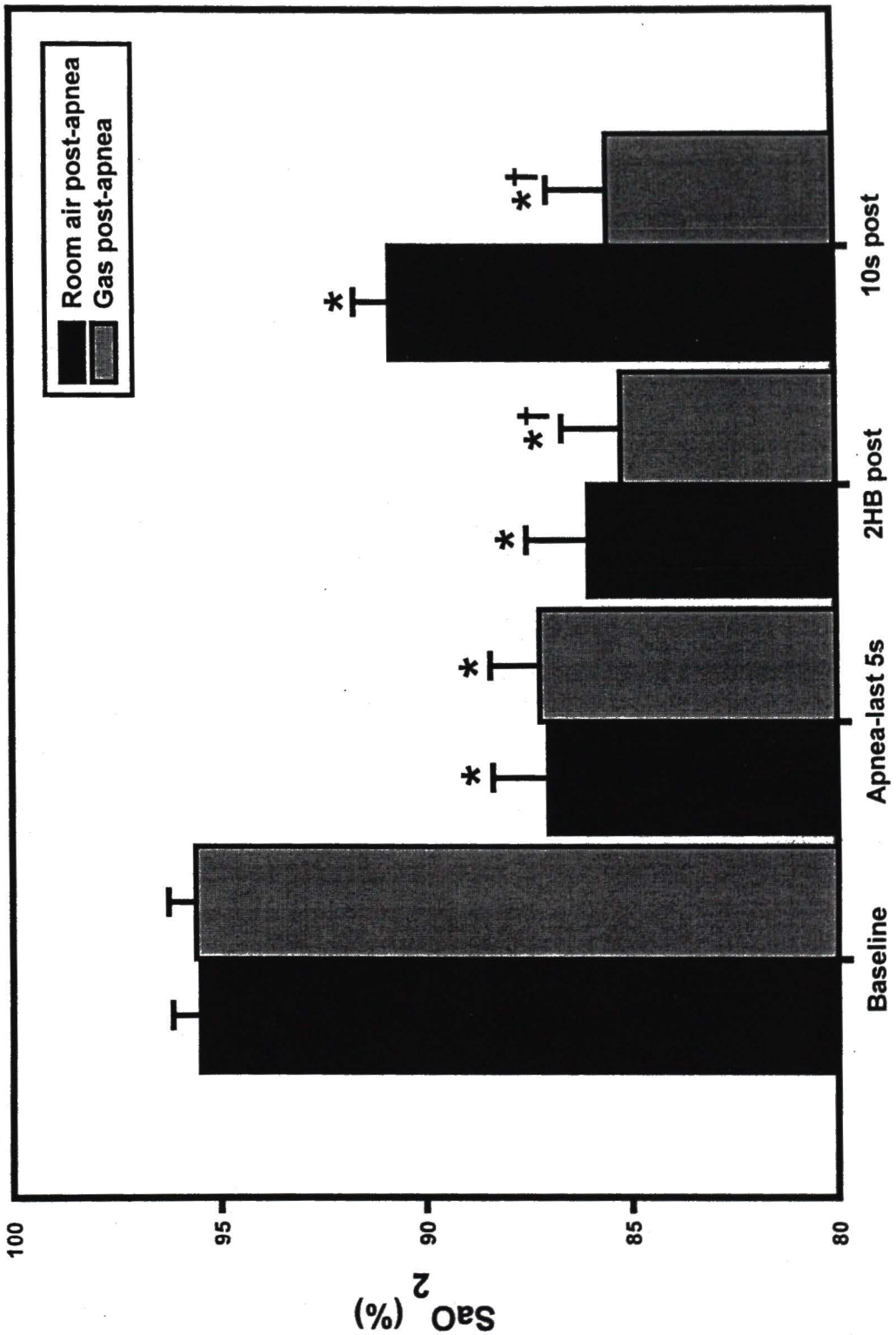
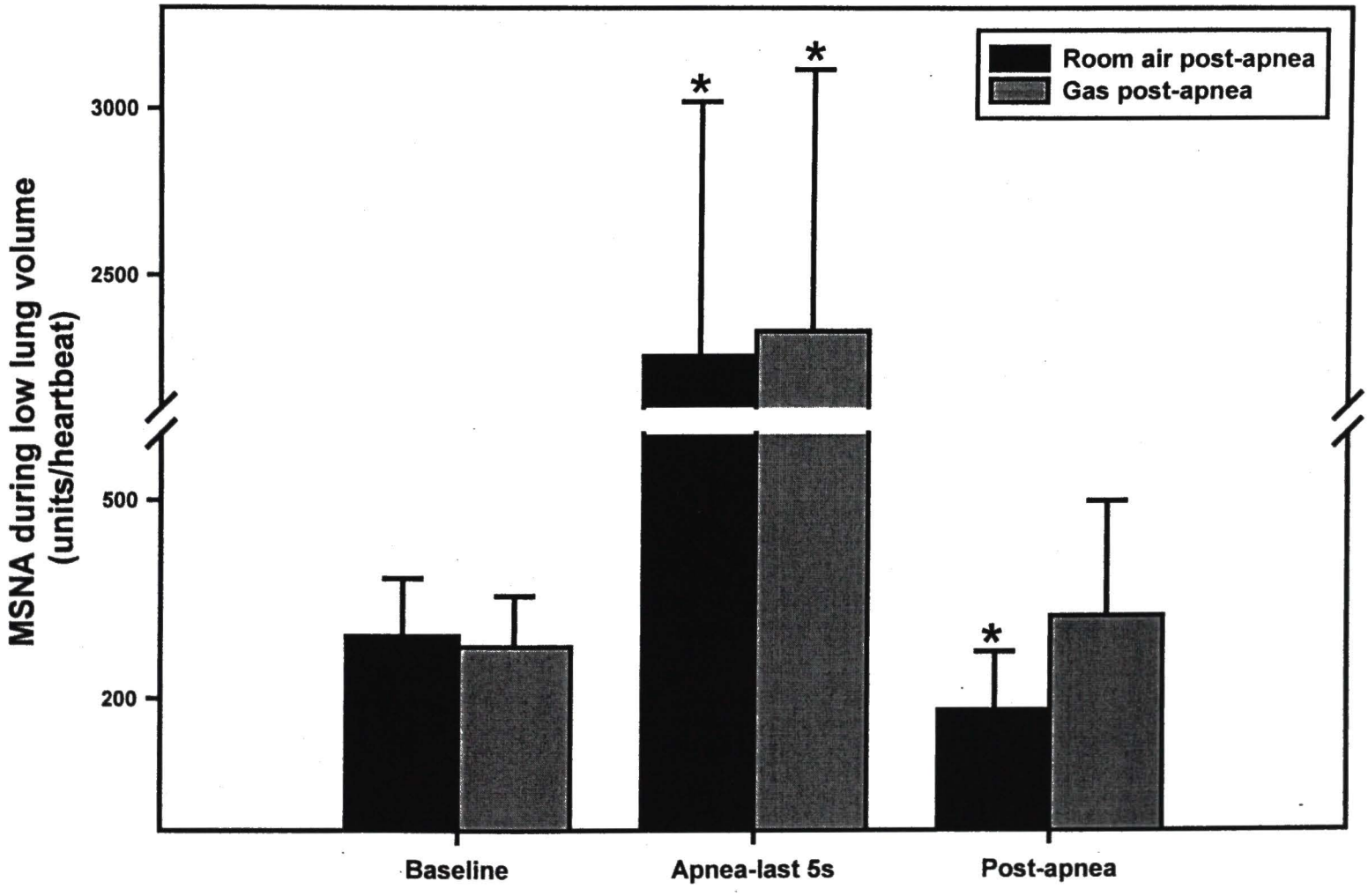

Room air post-apnea
Gas post-apnea
SaO_2 (%)
100
95
90
85
80
Baseline
Apnea-last 5s
2HB post
10s post
*
*
*†
*†

Figure 7


Room air post-apnea
Gas post-apnea
MSNA during low lung volume (units/heartbeat)
3000
2500
500
200
*
*
*
Baseline
Apnea-last 5s
Post-apnea

CHAPTER III

CAROTID BAROREFLEX FUNCTION DURING AND FOLLOWING VOLUNTARY APNEA IN HUMANS

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American Journal of Physiology

(Heart and Circulatory Physiology)

ABSTRACT

Muscle sympathetic nerve activity (MSNA) and arterial pressure increase concomitantly during apnea, suggesting possible overriding of arterial baroreflex sympathoinhibitory input to sympathoregulatory centers by apnea-induced sympathoexcitatory mechanisms. Apnea termination is accompanied by strong sympathoinhibition while arterial pressure remains elevated. Therefore, we hypothesized that carotid baroreflex control of MSNA would decrease during apnea and return upon apnea termination. MSNA and heart rate responses to –60 Torr neck suction (NS) were evaluated during baseline and throughout apnea. Responses to +30 Torr neck pressure (NP) were evaluated during baseline and throughout one minute post-apnea. Apnea did not affect the sympathoinhibitory or bradycardic response to NS ($P > 0.05$); however, while the cardiac response to NP was maintained post-apnea, the sympathoexcitatory response was reduced for 50 s ($P < 0.05$). These data demonstrate that carotid baroreflex control of MSNA is not attenuated during apnea. We propose a transient resetting of the carotid baroreflex-MSNA function curve during apnea, and that return of the function curve to baseline upon apnea termination partly explains the reduced sympathoexcitatory response to NP.

INDEX TERMS:

sympathetic nerve activity, heart rate, neck suction, neck pressure

INTRODUCTION

Muscle sympathetic nerve activity (MSNA) increases progressively during apnea and apnea termination is associated with an immediate and profound sympathoinhibition (11, 12, 16, 19, 22, 30, 34, 43). The mechanism(s) of the post-apneic sympathoinhibition is unclear; potential contributors include the normalization of blood gases, activation of the lung inflation reflex, baroreflex activation, and the return of central respiratory drive.

The importance of chemoreflex activation to the apnea-induced sympathoexcitation has been well demonstrated (11, 16, 19, 30). It follows that the post-apneic sympathoinhibition is therefore due, in part, to blood gas normalization; however, our laboratory has recently demonstrated that chemoreflex unloading is not important to the post-apneic sympathoinhibition relative to end-apnea (20). In addition, we have shown that the lung inflation reflex is not likely the primary mediator of post-apneic sympathoinhibition (20).

The apnea-induced sympathoexcitation leads to a significant increase in arterial pressure (11, 12, 15, 16, 19, 25, 34, 39, 43), which typically peaks post-apnea due to the latency of MSNA translating into increased vascular resistance and blood pressure (42). The rise in arterial pressure during apnea would be expected to inhibit MSNA via the arterial baroreflexes, yet MSNA greatly increases throughout apnea. Thus, the sympathoinhibitory input from the arterial baroreflexes appears to be overridden during apnea by sympathoexcitatory mechanisms such as the chemoreflex and lack of stimulation to the pulmonary stretch afferents. However, upon apnea termination, MSNA

is strongly inhibited (11, 12, 16, 34, 43), even when the chemoreflex stimuli are maintained (20). These findings suggest that the role arterial baroreceptors play in the dynamics of MSNA control may differ profoundly between the conditions of apnea and apnea termination. The balance of inputs to the sympathoregulatory centers of the medulla may be altered upon apnea termination, such that sympathoinhibitory input from the arterial baroreceptors predominate.

Therefore, the purpose of the present study was to determine whether carotid baroreflex function changes with apnea and apnea termination. Application of 5 s pulses of –60 Torr neck suction (NS) was performed during baseline and at the beginning, middle, and end of end-expiratory apnea to determine whether the sympathoinhibitory response of the carotid baroreflex diminished with apnea. Application of 5 s pulses of +30 Torr neck pressure (NP) was performed during baseline and throughout one minute of post-apneic recovery to assess the sympathoexcitatory response of the carotid baroreflex during post-apneic sympathoinhibition. We applied only a hypertensive stimulus during apnea because MSNA is so elevated that further increases would be difficult to observe; similarly, we applied only a hypotensive stimulus during post-apneic recovery because MSNA is very low post-apnea and further inhibition would be difficult to measure, as MSNA is often at or near zero. We hypothesized that carotid baroreflex control of MSNA would decrease during apnea and return upon apnea termination.

METHODS

This study was approved by the University of North Texas Health Science Center Institutional Review Board. Ten volunteers (3 women, 7 men, ages 21 to 30 years, weight: 75.7 ± 3.5 kg, mean ± SE) participated in the study after giving written, informed consent. All subjects completed a medical history/health questionnaire to establish good health. Women subjects tested negative for pregnancy and were not studied during menses to eliminate potential confounding effects of menses on fluid metabolism, blood volume, and cardiovascular function. Subjects were asked to abstain from vigorous exercise and alcohol for 24 hours prior to the study start time, and caffeine for 12 hours prior.

Measurements

Heart rate was obtained using a standard limb-lead ECG. Arterial blood pressure was measured non-invasively from beat-to-beat photoplethysmographic recordings at the finger (Finapres Blood Pressure monitor 2300, Ohmeda, Inc., Englewood, CO). This method has been validated against direct arterial pressure recordings (14, 23). A respiratory monitoring band was placed around the subject's abdomen (Grass Instruments, West Warwick, RI), allowing investigators to monitor breathing frequency and to insure that all apneas were performed at end-expiration and that all neck suction/pressure pulses were delivered at end-expiration. Pulse oximetry at the finger assessed oxygen saturation (Sa_{O_2}) (DS-100A Durasensor, Nellcor Puritan Bennett Inc.,

Pleasanton, CA). Subjects were also fitted with an airtight mouthpiece and 3-way Rudolph valve connected to a Douglas bag containing a gas mixture of 13% O_2/87% N_2, which subjects respired during baseline.

Muscle sympathetic nerve instrumentation. Efferent muscle sympathetic nerve activity (MSNA) was directly measured from the peroneal nerve at the popliteal fossa utilizing standard microneurographic techniques (1, 30). Two sterile tungsten microelectrodes (tip diameter 5-10 μm, 35 mm long, Frederick Haer and Co., Bowdoinham, ME, USA) were inserted; one was inserted into the peroneal nerve for recording of MSNA and the other served as a reference. Microelectrodes were inserted without local anesthesia since they are so small they do not cause appreciable pain when inserted, and because anesthesia might affect local nerve function. A signal processing system rectified and integrated the nerve signal, and amplified it approximately 9×10^4 times (University of Iowa Bioengineering, Iowa City, Iowa, USA). Pulse synchrony of bursts and reproducible activation during apnea, and lack of response to skin stroking or startle stimuli, confirmed recording of muscle, and not skin, sympathetic nerve activity.

Carotid baroreflex function. Investigators adjusted carotid baroreceptor activation and deactivation via the delivery of suction and pressure to an airtight chamber enclosing the anterior portion of the neck. This method of assessing carotid baroreflex function was first described by Ernsting and Parry (8) and later simplified and improved by Eckberg and colleagues (2). Subjects were fitted with a cushioned, malleable lead collar which encased the anterior two-thirds of the neck. Pressure of +30 Torr and suction of –60 Torr were generated by a manually controlled, variable pressure source

and delivered through large bore two-way solenoid valves (Asco, Florham Park, NJ) to the neck chamber. A pressure transducer (Validyne Engineering Corporation, Northridge, CA) measured neck chamber pressure.

The responses to NS/NP pulses are affected by the point in the cardiac and respiratory cycles in which they are delivered (2-4, 6, 7, 17, 36); thus, the timing of NS/NP pulses in relation to these two cycles was kept constant throughout the study. The timing of each NS/NP pulse in relation to the cardiac cycle was computer-controlled such that each pulse was initiated precisely 50 ms after the R-wave of the ECG and was maintained for 5 s. The timing of each NS/NP pulse in relation to the respiratory cycle was controlled manually by an investigator who observed the respiratory signal; when subjects reached approximately the last fourth of expiration, the computer-controlled neck pulse was activated. Only those neck pulses actually initiated during end-expiration (i.e., the nadir in the respiratory signal) were included in the data analyses.

Protocol

These studies were performed with subjects in the supine position. Subjects were instrumented for recording of heart rate, blood pressure, respiratory activity, and Sa_{O_2}, and were fitted with a neck collar appropriate for their size. Practice NS/NP pulses were then performed to verify both a tight seal and encasement of the carotid bifurcation within the neck chamber, based on heart rate responses. The collar was then removed, for comfort's sake, while the subject was instrumented for recording of MSNA. The neck collar was then re-applied in the same position and the subject fitted with the

mouthpiece, and normal resting respiratory frequency was determined. A computer sound file was then used to guide the subject to maintain respiratory frequency at the resting frequency throughout the protocol. Eight repetitions of the following procedure were then performed: i) one minute of baseline breathing of a 13% O_2/87% N_2 gas mixture (to maximize O_2 desaturation with apnea), ii) a 20 s end-expiratory apnea, and iii) one minute of recovery. Subjects controlled respiratory frequency during baseline and recovery and were asked to maintain a consistent tidal volume during baseline, but were allowed in increase tidal volume as necessary following apnea. Neck pulses were randomly delivered as follows during six of the repetitions: i) NS/NP approximately 35 s into baseline, ii) NS during either the first (1-6 s; A1), second (8-13 s; A2), or last (14-19 s; A3) third of apnea, and iii) NP during the first post-apneic expiration and then repeatedly throughout recovery, with a minimum of 5 s between the termination of one pulse and the initiation of the next. Thus, three NS and three NP pulses were performed during baseline, two NS pulses were performed at each third of apnea (A1-A3), and up to six NP pulses were obtained for each of the following time points in recovery: 1-10 s (recovery 1; R1), 11-20 s (recovery 2; R2), 21-30 s (recovery 3; R3), 31-40 s (recovery 4; R4), 41-50 s (recovery 5; R5), and 51-60 s (recovery 6; R6). If error in NS/NP timing occurred, additional repetitions were performed as necessary so that at least two correctly-timed NS/NP pulses were obtained for each time point for each subject. Only NS was applied during apnea because MSNA is so high that additional increases due to NP would be difficult to measure; likewise, only NP was delivered during recovery because post-apneic sympathoinhibition would make responses to NS difficult to observe

and measure since MSNA is often at or near zero. The remaining two repetitions served as controls and consisted of the same procedure of baseline, apnea, and recovery, but with no delivery of NS/NP pulses. These two controls were placed randomly within the eight repetitions.

Data analyses

MSNA and heart rate responses to NS/NP were averaged for each time point for each subject. Timing of MSNA bursts was adjusted to correct for nerve conduction delay(10). Only MSNA occurring within 2.5 s of stimulus initiation was used in the quantification of the MSNA responses, as it has been shown that the MSNA response to carotid baroreceptor loading and unloading is transient, lasting only 1-2 bursts and then returning to baseline activity, in spite of continued baroreceptor loading/unloading (7, 24, 40, 41). MSNA was calculated as total activity (sum of areas of bursts) rather than burst frequency, as burst amplitude and duration are greatly increased during apnea and these increases would not be reflected in burst frequency. Each value of total activity was multiplied by 10,000 for the convenience of working with whole numbers, and calculated as both units/heartbeat and units/s. MSNA data analyses yielded the same results for MSNA/heartbeat and MSNA/s, so MSNA is reported here only as total activity/heartbeat. Due to high inter-individual variability in nerve activity, MSNA was normalized such that each subject's baseline activity equaled 100 units/heartbeat, and MSNA at all other time points was quantified as a percent of this baseline.

To determine the MSNA response to NS/NP, MSNA associated with the first 2.5 s of NS/NP was compared to MSNA during the time-matched, respiratory-cycle matched controls in which no neck pulse was delivered. For example, if a subject's average time of NS delivery during baseline was 37 s into baseline, the investigator would begin the control files at the end-expiratory period nearest 37 s into baseline. Change in MSNA due to NS/NP was thus calculated as average MSNA during the first 2.5 s of NS/NP minus average MSNA during the time-matched, respiratory-matched controls. The reason for using the time-matched control files to determine MSNA responses rather than using MSNA just prior to NS/NP delivery is because physiological variables are constantly changing during apnea: for example, MSNA 10 s into apnea is not an appropriate control for MSNA during NS delivered 13 s into apnea, because between 10 and 13 s into apnea, Sa_{O_2} falls, arterial pressure increases, and MSNA increases. Thus, in these circumstances, a time-matched control file is more appropriate. Heart rate responses were similarly calculated as minimum and maximum heart rate during NS and NP, respectively, minus average heart rate during time- and respiratory-matched controls.

Statistical analyses were divided into two parts: carotid baroreflex responsiveness to NS at baseline and throughout apnea, and carotid baroreflex responsiveness to NP at baseline and throughout recovery. Two-way repeated-measures analysis of variance (ANOVA) was used to determine the effect of time and NS/NP on dependent variables, and whether an interaction existed between the two factors. If significance was found, *post hoc* multiple comparison Tukey tests were performed to determine where specific

differences existed. All statistical analyses were performed at a significance level (α) of 0.05. All data are expressed as means ± SE.

RESULTS

Ten volunteer subjects were enrolled in the study, but the data for two subjects were not analyzed due to technical difficulties. A sample tracing of one subject's raw data is depicted in Figure 1. In the upper graph (1A), responses to NS delivered during the last third of apnea (A3) and NP delivered during the first post-apneic expiration (R1) are shown. The lower graph (1B) shows a time-matched control of the same subject. During the last third of apnea, MSNA bursts occurred with every heartbeat and with increasing amplitude and duration. Application of NS completely inhibited a single MSNA burst (see arrow), demonstrating the strength and brevity of the MSNA response during apnea. Apnea termination was associated with an immediate and profound sympathoinhibition. Application of NP during the first post-apneic expiration (R1) was unable to elicit a sympathoexcitatory response. This post-apneic attenuation of the MSNA response to NP was seen in all subjects.

Effect of apnea and apnea termination on MSNA responses to NS/NP (Figures 2 and 3). Apnea increased MSNA above baseline activity (P = 0.02), with the only specific difference occurring between baseline and A3 (P = 0.015; Figure 2). NS decreased MSNA during baseline and apnea (P = .003), and there was no interaction between time

and NS (P = 0.127). Therefore, in spite of the strong sympathoexcitatory effect of apnea, carotid baroreflex control of MSNA was preserved.

Post-apneic recovery was associated with decreased carotid baroreflex control of MSNA (Figure 3). Two-way repeated-measures ANOVA showed a significant interaction between time and NP on MSNA (P = 0.036). NP significantly increased MSNA at baseline (P = 0.006), but this sympathoexcitatory effect of NP was not present following apnea termination (R1 through R5). Only during the last 10 s of post-apneic recovery (R6) was the MSNA response to NP restored (P = 0.004); however, the MSNA response showed a clear trend for recovery at time points R4 and R5.

Effect of apnea and apnea termination on heart rate responses to NS/NP (Figures 4 and 5). NS significantly affected heart rate (P = 0.014), as did time (P = 0.043; Tukey: A1 vs. A3, P = 0.041). There was no interaction between the two factors (P = 0.107). Thus, as with MSNA, apnea did not affect the heart rate response to NS.

A significant interaction between time and NP existed in heart rate responses during post-apneic recovery (P = 0.028). However, *post hoc* analyses determined that NP significantly increased heart rate at all time points (baseline through R6, all P < 0.01). Thus, unlike MSNA, carotid baroreflex control of heart rate was preserved following apnea termination.

DISCUSSION

We hypothesized that carotid baroreflex control of MSNA diminishes with apnea, as MSNA and arterial pressure concomitantly increase during apnea. In addition, due to the sympathoinhibition post-apnea while arterial pressure remains elevated, we hypothesized that effectiveness of carotid baroreflex control of MSNA returns upon apnea termination. However, both hypotheses were refuted. Carotid baroreflexes maintained their ability to decrease MSNA in response to –60 Torr NS throughout apnea, even during the last third of apnea when strong sympathoexcitatory stimuli were present. Following apnea termination, carotid baroreflexes were unable to elicit a significant increase in MSNA in response to +30 Torr NP; however, this effect was transient and normal baroreflex response had returned by the end of the post-apneic recovery minute. In contrast to the sympathetic arm of the carotid baroreflex, cardiac responses to NS/NP were preserved both during apnea and following its termination. These heart rate responses to brief NS/NP are predominately mediated by parasympathetic nerve activity (5).

Resetting of the carotid baroreflex-MSNA (CBR-MSNA) function curve has been demonstrated with exercise (9). Fadel *et al.* showed that the CBR-MSNA function curve reset to the higher arterial pressure and higher MSNA induced by exercise (i.e., a rightward and upward shift in the curve), but that the properties of the CBR-MSNA function curve were unchanged. While the current study did not attempt to model the entire CBR-MSNA function curve, we propose that a similar resetting is occurring with

apnea (Figure 6). During baseline resting conditions, the operating point is located near the onset mean arterial pressure (MAP) for sympathoexcitation (24)(point B in Figure 6). Baseline application of –60 Torr NS produced essentially complete sympathoinhibition in our subjects (98 ± 2 % decrease in MSNA); however, the absolute change in MSNA was relatively small, due to low baseline sympathetic tone (Figure 2). Apnea caused approximately a 10 mm Hg increase in MAP. If the CBR-MSNA function curve did not reset during apnea, the rise in MAP would be expected to significantly reduce MSNA (28). However, at end-apnea, MSNA increased over ten-fold, consistent with a rightward and upward resetting of the CBR-MSNA function curve (Figure 6). NS of –60 Torr during the last third of apnea produced a significant sympathoinhibitory response, with the absolute decrease in MSNA being even greater than at baseline due to the high MSNA during apnea (Figure 2). These results also suggest that the operating point of the CBR-MSNA function curve may have moved leftward (i.e., further from saturation and closer to threshold), as hypothetically presented as point A3 in Figure 6. If such a resetting of the CBR-MSNA function curve and movement of the operating point on the CBR-MSNA function curve do occur with apnea, then baroreflex control of MSNA shifts from being better poised to handle a hypotensive stimulus during baseline to being better poised to handle a hypertensive stimulus during apnea.

During the first 10 s post-apnea, MAP remained elevated and MSNA was almost completely inhibited. Therefore, we further propose that the CBR-MSNA function curve reset back to baseline upon apnea termination, with the operating point hypothetically at point R1 in Figure 6. This would partially explain the reduced sympathoexcitatory

response to NP at R1, as the operating point would now be located on the relatively flat portion of the curve. However, this cannot fully explain the post-apneic reduction in CBR-MSNA response to a hypotensive stimulus. Sympathoexcitatory response to +30 Torr NP was clearly reduced at recovery points R1 through R3, showed a trend for recovery at R4 and R5, and had regained baseline function by R6 (Figure 3). If the location of the operating point on the flat part of the function curve were the sole explanation for the reduced baroreflex responsiveness, then MAP would be expected to follow a similar time course of recovery as baroreflex function, returning to baseline pressures at approximately R6. However, MAP had returned to pressures not significantly different from baseline by R2 (Figure 7).

Van de Borne and colleagues have demonstrated that isocapnic, increased-tidal volume breathing leads to an attenuation of arterial baroreflex control of MSNA (38). In addition, Macefield and Wallin noted that the duration of sympathoinhibition and abnormal respiration post-apnea were similar, suggesting that ventilation is importantly involved in post-apneic sympathinhibition (18). In the current study, subjects controlled respiratory frequency throughout the protocol, but were allowed to increase tidal volume as necessary post-apnea. Thus, elevated tidal volume could potentially explain the reduced MSNA response to NP post-apnea. Unfortunately, we do not have tidal volume data; only respiratory movements were recorded via a respiratory monitoring band. Therefore, we cannot determine whether increased tidal volume and decreased CBR-MSNA function were correlated in our subjects. Nevertheless, our data demonstrate that baroreflex control of MSNA is attenuated immediately post-apnea; this is consistent with

resetting of the reflex back to the pre-apnea state (Figure 6) and may also involve modulation by ventilation or other inputs such as the cardiopulmonary baroreceptors. Regardless, the abruptness of the sympathoinhibition at apnea termination (20, 43) strongly implies that the altered baroreflex control during recovery from apnea is mediated by central nervous system modulation of the reflex and not modulation at the baroreceptors.

The bradycardic response to –60 Torr NS was not altered during apnea, nor was the tachycardic response to +30 Torr NP altered during post-apneic recovery. A previous study determined that hypoxia decreased cardiac baroreflex sensitivity (44); we did not find a change in cardiac baroreflex function with decreased Sa_{O_2}, which fell to 91 ± 1% at the A3 time point. However, differences exist between the two studies which could explain the different results. Ziegler *et al.* (44) measured cardiac baroreflex sensitivity during breathing of a 15% O_2 gas mixture which decreased Sa_{O_2} to 90% or below in their subject groups, comparable to Sa_{O_2} in our subjects at end-apnea. However, their subjects were respiring while ours were in apnea. Hypoxia during respiration significantly increases heart rate (11, 13, 26, 27, 31-33, 37, 45) and did so in their study, but hypoxia during apnea decreases heart rate (45). During the last third of apnea our subjects had slightly, but not significantly, decreased heart rates compared to baseline. Thus, the hypoxia-induced tachycardia present in the subject population of Ziegler *et al.* (44) could have counteracted the bradycardic response to phenylephrine-induced increases in pressure.

The decreased MSNA response to NP post-apnea but maintenance of the heart rate response indicates a disassociation between the sympathetic and cardiac parasympathetic branches of the carotid baroreflex. Wallin and Eckberg have suggested that central nervous system processing of arterial baroreceptor input is different for parasympathetic and sympathetic branches of the reflex, based on their observation of different degrees and time courses of adaptation of these two branches to the same baroreceptor stimulus (41). Additionally, Narkiewicz *et al.* reported impairment of the sympathetic but not the cardiac branch of the arterial baroreflex in obstructive sleep apnea patients, independent of hypertension, obesity, and age (21). Interestingly, the MSNA baroreflex impairment was selective for hypotensive but not hypertensive stimuli. We also found impairment of the sympathetic but not the cardiac response to a hypotensive stimulus; however, this impairment was transient and occurred post-apnea in healthy individuals. Nonetheless, it is interesting to note the similarities in the results of these two studies, and it raises the possibility that the transient post-apneic reduction in CBR-MSNA responsiveness to a hypotensive stimulus may eventually translate into a permanent baroreflex impairment in patients experiencing repetitive apneas each night.

Study limitations

Subjects were breathing a 13% O_2/87 % N_2 gas mixture during baseline data collection. As discussed above, Ziegler *et al.* have demonstrated that breathing a hypoxic gas reduces cardiac-baroreflex sensitivity (44). However, during baseline baroreflex measurements in the current study, subjects' Sa_{O_2} averaged 96 ± 1%, essentially

equivalent to the Sa_{O_2} during normoxia in the study of Ziegler *et al.* A reduction in baroreflex sensitivity was noted in their subjects at Sa_{O_2} values of 90% and below. Thus, it is doubtful that baroreflex function was affected by the mild oxygen desaturation during baseline.

During baseline and post-apneic recovery, NS/NP pulses were delivered during respiration, while during apneic application of NS, no respiration was present. Responses to carotid baroreceptor stimulation have been shown to be affected by the point in the respiratory cycle in which the stimuli are applied (4, 6, 7, 36). To minimize these effects, NS/NP pulses delivered during respiration were applied only at end-expiration, as all apneas were held at end-expiration. However, it remains possible that the difference in respiratory state between baseline and apnea affected the responses to NS. This seems unlikely, however, because no change in carotid baroreflex function was found from baseline throughout apnea.

The current study assessed responses to only a hypertensive stimulus during apnea and to only a hypotensive stimulus during post-apneic recovery. The MSNA responses are consistent with resetting of the carotid baroreflex function curve as proposed in Figure 6; however, we did not model the complete reflex function curve at each time point. Thus, at present this mechanism is only speculative.

Lastly, application of NS/NP tests carotid baroreflex function only. MAP remained elevated during the first 10 s post-apnea and was still slightly, though not significantly, elevated during the second 10 s post-apnea. Therefore during post-apnea time points R1 and R2, sympathoinhibitory input from the aortic baroreflex was

counteracting the sympathoexcitatory input from the carotid baroreflex during application of NP. This could partly explain the decreased MSNA response to NP post-apnea. Studies have suggested that when the aortic and carotid baroreceptors are sending conflicting input to sympathoregulatory centers, the loaded baroreceptors dominate the response (29, 35). However, in neither study were the loaded baroreceptors the aortic baroreceptors, as is the case post-apnea in the current study. As discussed above, MAP has returned to baseline by R3 (and is not significantly different from baseline at R2); thus, sympathoinhibitory input from aortic baroreceptors cannot explain the decreased MSNA response to NP at time points R3 through R5.

In conclusion, we found that sympathoinhibitory responses to –60 Torr NS do not diminish with apnea, while sympathoexcitatory responses to +30 Torr NP are reduced following apnea termination but recover within one minute. In addition, heart rate responses to –60 Torr NS and +30 Torr NP are not affected by apnea or its termination, respectively. We propose a rightward and upward resetting of the carotid baroreflex-MSNA function curve with apnea, similar to that seen with exercise (9), as well as shifting of the operating point away from saturation and towards threshold. Return of the carotid baroreflex-MSNA function curve to baseline upon apnea termination may partly explain the reduced MSNA response post-apnea; however, because MAP has returned to baseline before the sympathoexcitatory response has returned to baseline, this does not fully explain the reduced MSNA response to NP during post-apneic recovery.

ACKNOWLEDGEMENTS

The authors thank the subjects for their cooperation and contribution to this work. This research was supported by NIH grant HL-49266, for which we are grateful.

This research was submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy for Nicolette Muenter Swift.

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FIGURE LEGENDS

Figure 1. Sample tracing of raw data from a single representative subject. Nerve signal has not been corrected for conduction delay. Graph A depicts responses to –60 Torr NS delivered during the last third of apnea (A3) as well as responses to +30 Torr NP delivered during the first post-apneic expiration (R1). During apnea, MSNA bursts occurred with each heartbeat and NS completely abolished a single burst (bold arrow). NP was unable to elicit an MSNA response post-apnea. Graph B depicts the same subject during a time-matched control, in which no NS/NP pulses were delivered. MSNA bursts occurred with every heartbeat during the last third of apnea, and MSNA was completely inhibited upon apnea termination. NCP = neck chamber pressure.

Figure 2. MSNA response to –60 Torr NS at baseline and throughout apnea (A1-A3). Apnea significantly increased MSNA and NS significantly reduced MSNA, but no interaction existed between the two. Thus, although apnea caused a significant increase in MSNA, this did not affect the sympathoinhibitory response to NS. *Significantly less than time-matched control ($P < 0.05$). †Significantly greater than baseline ($P < 0.05$).

Figure 3. MSNA response to +30 Torr NP at baseline and throughout post-apneic recovery (R1-R6). There was a significant interaction between time and NP. *Post hoc* multiple comparison Tukey analyses revealed that NP significantly increased MSNA at baseline and R6, but not at R1 through R5. Thus, transitory attenuation of carotid

baroreflex control of MSNA appears to occur post-apnea. *Significantly greater than time-matched control ($P < 0.05$).

Figure 4. Heart rate response to –60 Torr NS at baseline and throughout apnea (A1-A3). Time and NS significantly affected heart rate, but no interaction existed between the two. Thus, apnea did not affect the bradycardic response to NS. *Significantly less than time-matched control ($P < 0.05$).

Figure 5. Heart rate response to +30 Torr NP at baseline and throughout post-apneic recovery (R1-R6). There was a significant interaction between time and NP. *Post hoc* multiple comparison Tukey test revealed that NP significantly increased heart rate at all time points. Thus, carotid baroreflex control of heart rate was preserved following apnea termination. *Significantly greater than time-matched control ($P < 0.05$).

Figure 6. Hypothetical resetting of the carotid baroreflex-MSNA function curve with apnea. Solid line represents the function curve during baseline; dashed line represents function curve during apnea, reset rightward and upward. Point B indicates the operating point during baseline conditions; point A3 indicates the operating point during the last third of apnea, with a shift away from saturation and towards threshold. Immediately post-apnea, we propose that the curve has reset back to baseline, with the operating point now at point R1, as MAP is still elevated while MSNA has decreased to nearly zero.

Figure 7. Mean arterial pressure (MAP) at baseline and throughout recovery. MAP had returned to levels not significantly different from baseline within 20 s of apnea termination (R2). *Significantly greater than all other time points ($P < 0.05$).

Figure 1

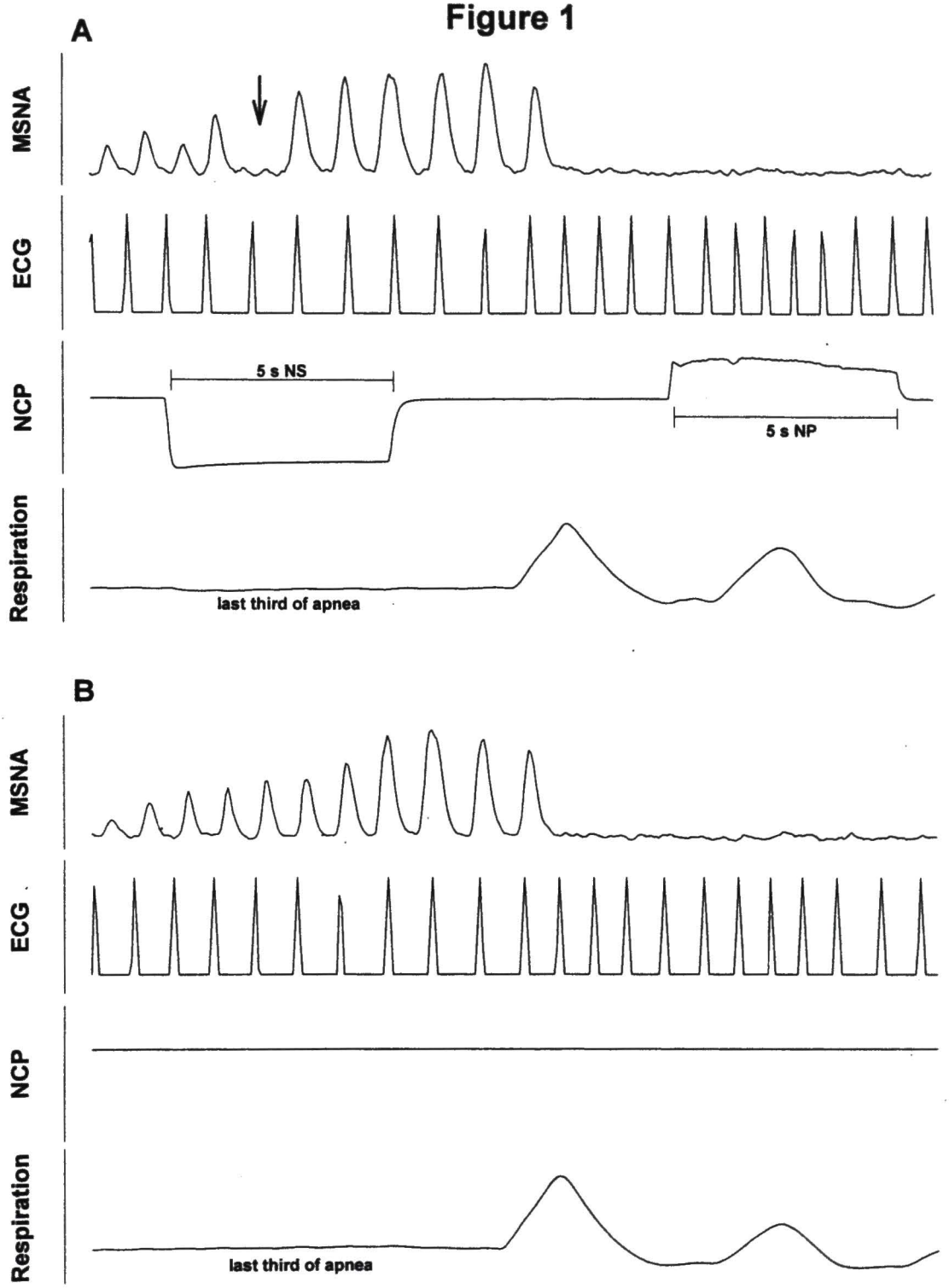

A
MSNA
ECG
NCP
Respiration
5 s NS
5 s NP
last third of apnea
B
MSNA
ECG
NCP
Respiration
last third of apnea

Figure 2

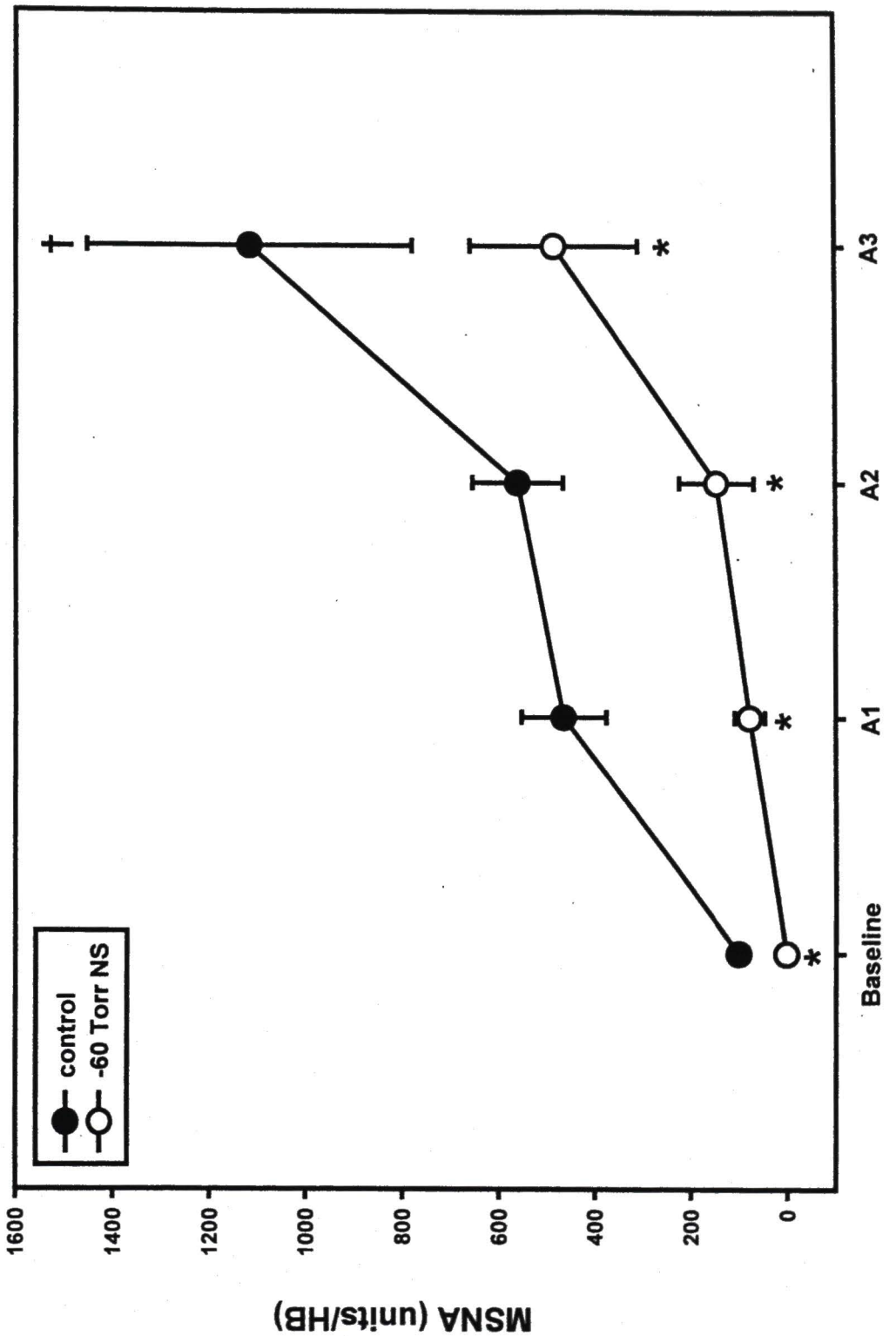

MSNA (units/HB)
1600
1400
1200
1000
800
600
400
200
0
control
-60 Torr NS
Baseline
A1
A2
A3
*
*
*
*
†

Figure 3

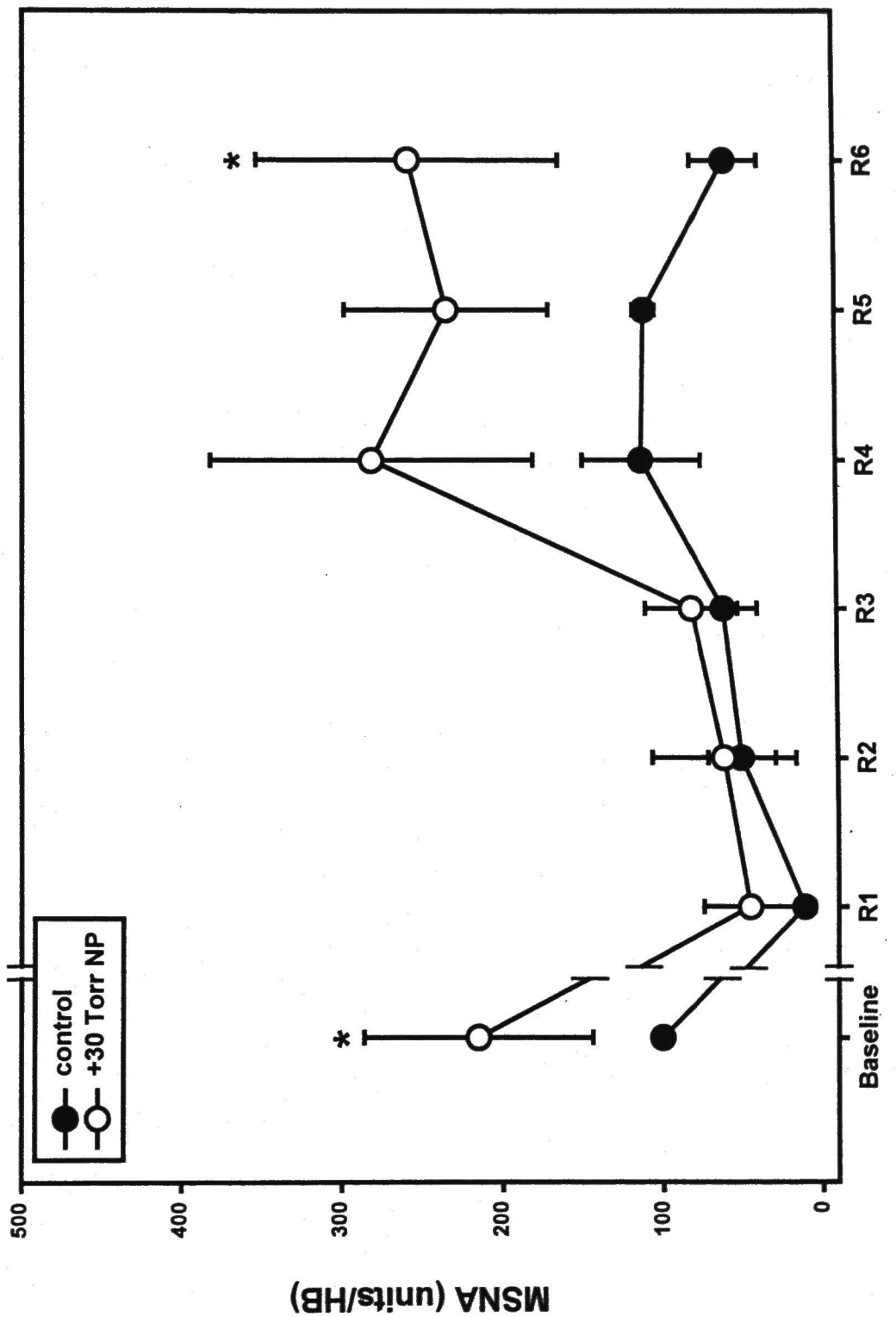

MSNA (units/HB)
500
400
300
200
100
0
control
+30 Torr NP
*
*
Baseline
R1
R2
R3
R4
R5
R6

Figure 4

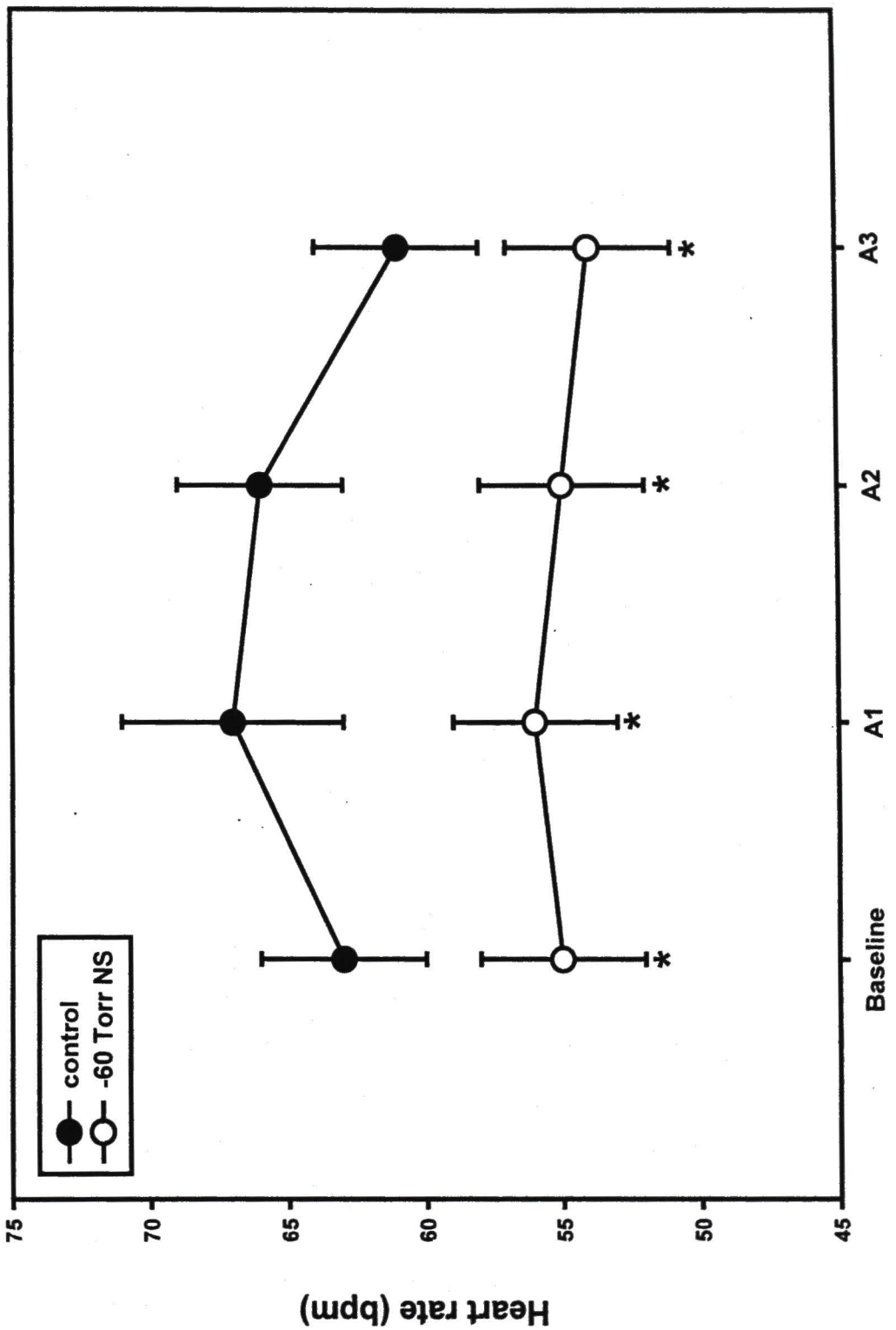

Heart rate (bpm)
75
70
65
60
55
50
45
control
-60 Torr NS
Baseline
A1
A2
A3
*
*
*
*

Figure 5

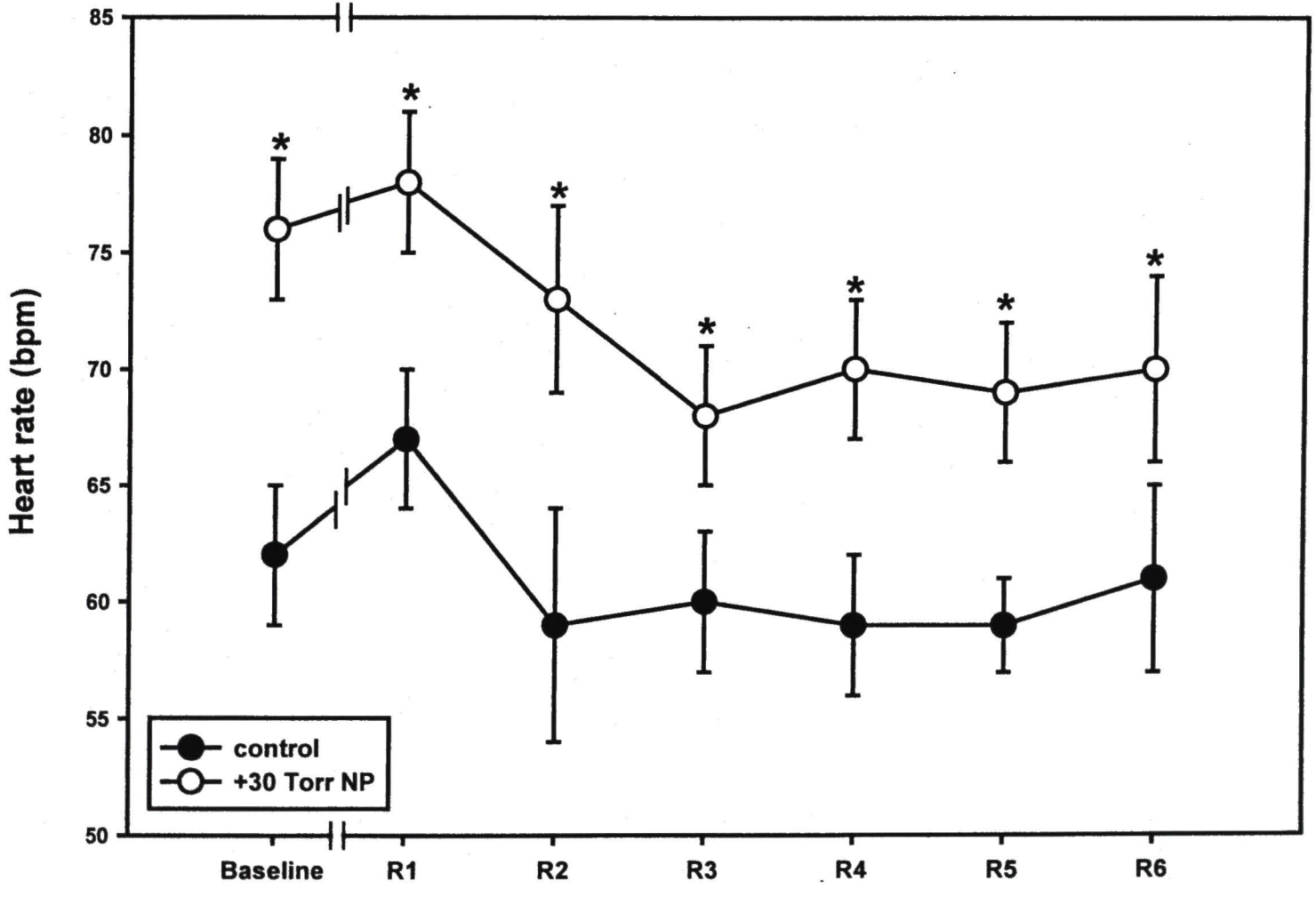

Heart rate (bpm)
85
80
75
70
65
60
55
50
*
control
+30 Torr NP
Baseline
R1
R2
R3
R4
R5
R6

Figure 6

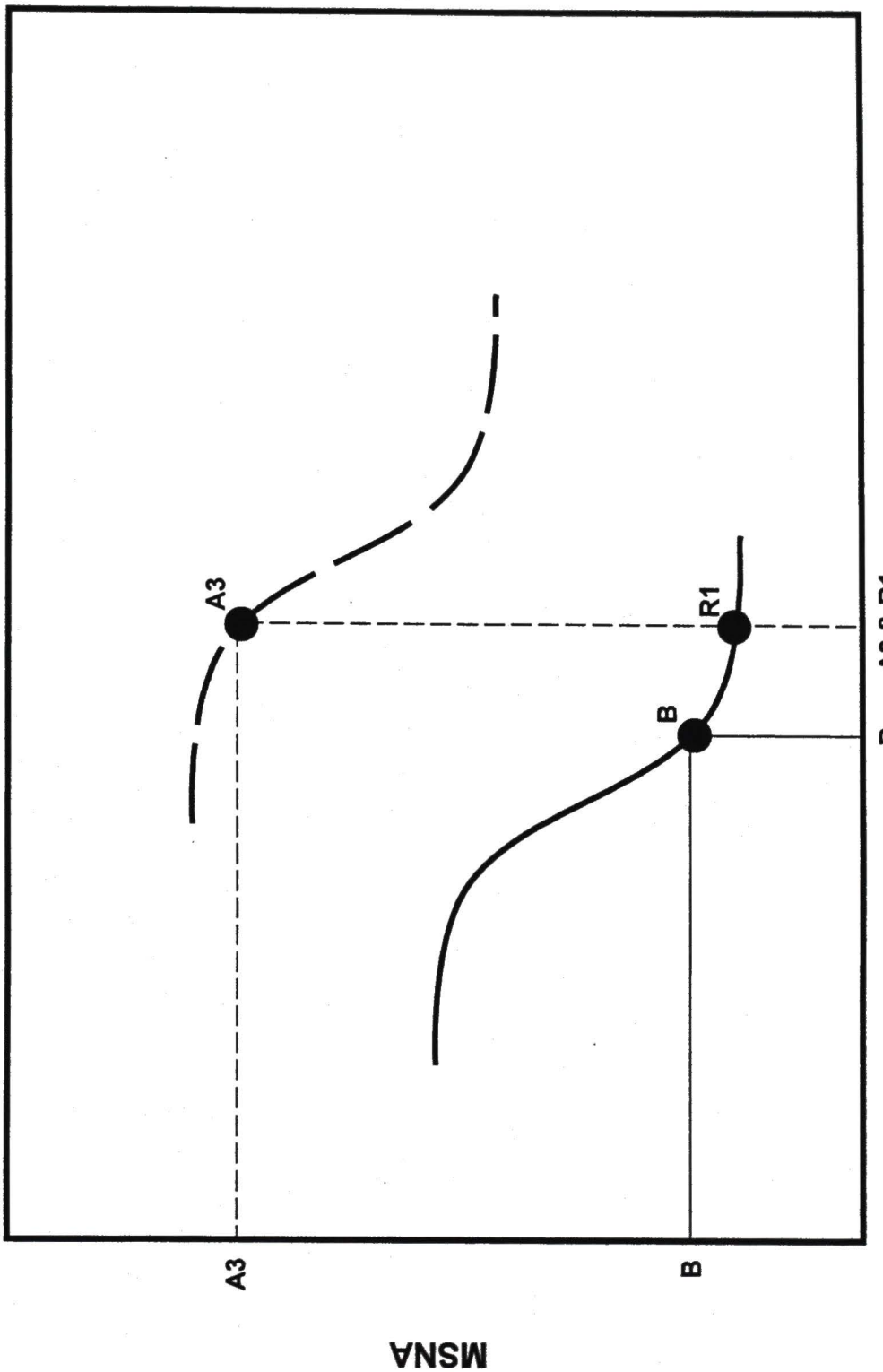
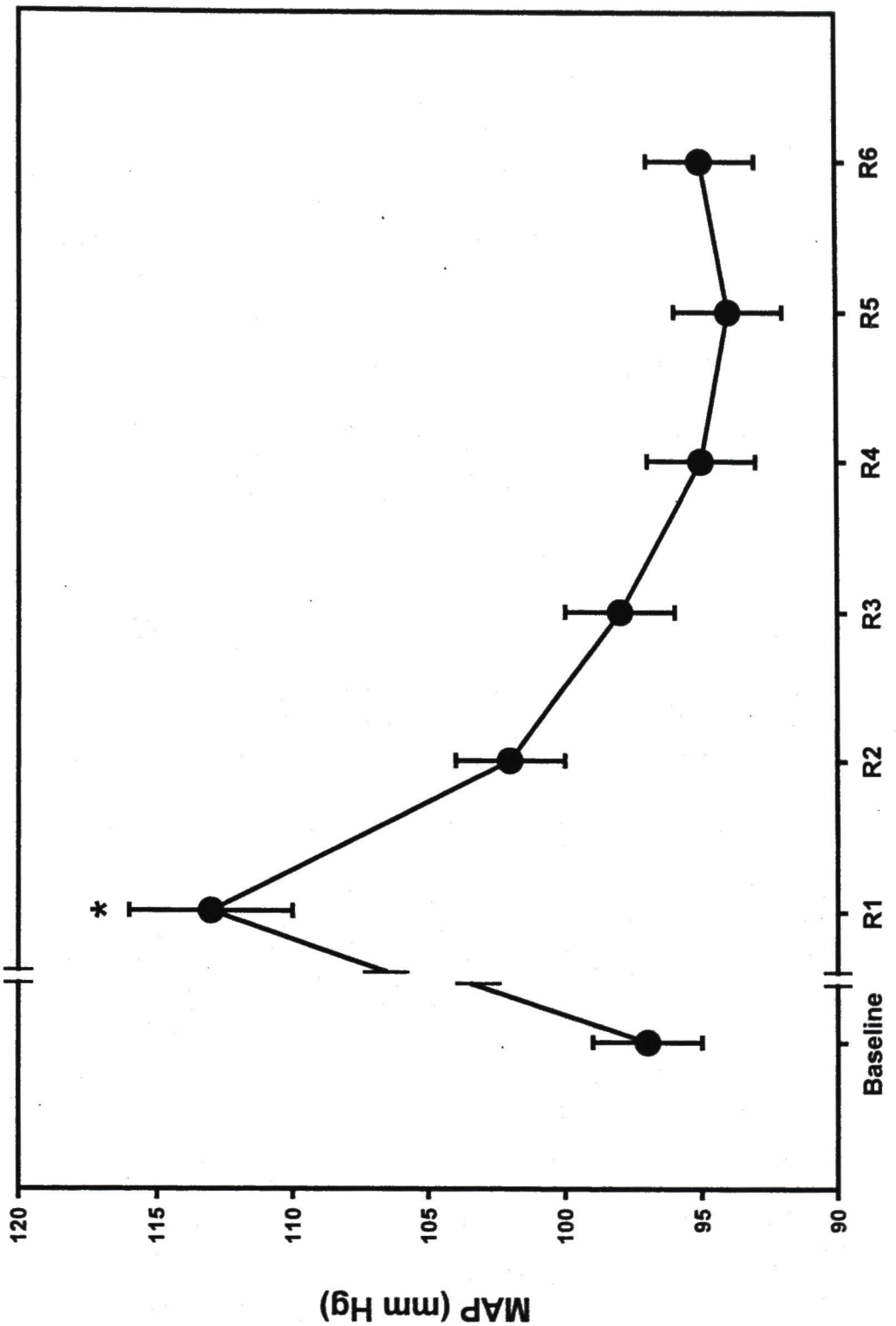


Figure 7



CHAPTER IV

CONCLUSIONS

The results of the current studies demonstrate that neither the normalization of blood gases nor the activation of the lung inflation reflex post-apnea importantly contributes to the profound and immediate sympathoinhibition observed upon apnea termination. Additionally, these studies indicate that carotid baroreflex control of muscle sympathetic nerve activity (MSNA) is preserved during apnea; however, at apnea termination the carotid baroreflex control of MSNA is attenuated.

The first study demonstrated that MSNA decreased from extremely high end-apnea activities (over ten times baseline activity) to activities at or below baseline upon apnea termination, regardless of chemoreflex unloading. Thus, the normalization of blood gases is not the primary mediator of post-apneic sympathoinhibition relative to end-apnea. However, post-apneic MSNA was significantly less than baseline pre-apneic MSNA only when blood gases were allowed to normalize; thus, normalization of blood gases does play a role in sympathoinhibition relative to baseline. Furthermore, the fact that virtually all post-apneic MSNA bursts occurred during the low lung volume phase of respiration indicates that the lung inflation reflex was operative post-apnea. Nonetheless, although the lung inflation reflex may contribute to the immediate post-apneic sympathoinhibition relative to baseline, it does not appear to be the primary mediator of the sustained sympathoinhibition relative to end-apnea, as this persists even during the

low lung volume phase of respiration, when sympathoinhibitory input from the lung inflation reflex is minimal.

The second study demonstrated that the sympathoinhibitory and bradycardic responses to –60 Torr neck suction (NS) were maintained throughout apnea; conversely, the sympathoexcitatory response to +30 Torr neck pressure (NP) was attenuated for nearly one minute post-apnea while the tachycardic response was maintained. Thus, apnea does not affect carotid baroreflex control of MSNA or heart rate, while apnea termination attenuates carotid baroreflex control of MSNA, but does not affect carotid baroreflex control of heart rate. Based on the simultaneous increase in arterial pressure and MSNA during apnea, as well as the preservation of carotid baroreflex control of MSNA, we propose that the carotid baroreflex-MSNA function curve resets in the rightward and upward direction during apnea and that the operating point moves away from saturation and towards threshold. Return of the function curve to baseline upon apnea termination may partly explain the reduced MSNA response to NP post-apnea; however, because arterial pressure has returned to baseline before the sympathoexcitatory response has returned to baseline, this cannot fully explain the reduced MSNA response during post-apneic recovery. Some other sympathoinhibitory mechanism active post-apnea must be tempering the carotid baroreflex-mediated sympathoexcitatory response to a hypotensive stimulus; it is unclear what this mechanism is, although it seems likely that it originates within the central nervous system.

CHAPTER V

SUGGESTIONS FOR FUTURE RESEARCH

The current studies provide evidence against chemoreflex unloading and activation of the lung inflation reflex as being the primary mediators of post-apneic sympathoinhibition. In addition, while the arterial baroreflexes appear to be playing a role, clearly some other sympathoinhibitory mechanism associated with apnea termination is importantly contributing. However, it remains unclear what this mechanism is. Therefore, additional research is needed to further our understanding of control of muscle sympathetic nerve activity (MSNA) during apnea and following its termination. Potential investigations which would further support the current research are listed below.

I. To further define the role of the lung inflation reflex in post-apneic sympathoinhibition, apneas could be performed in lung transplant patients, who lack an intact lung inflation reflex. If post-apneic sympathoinhibition is observed in these patients and is similar in magnitude to normal, healthy individuals, this would provide further evidence that the lung inflation reflex is not the primary mediator of post-apneic sympathoinhibition. A potential limiting factor of this type of study would be the health status of the lung transplant patients, which could affect their autonomic balance.

II. An experiment utilizing several different neck chamber pressures during and following apnea would enable investigators to model the carotid baroreflex-MSNA function curve. Thus, resetting of the function curve as well as movement of the operating point along the curve could be defined. In addition, it would be interesting to see if a stronger neck pressure stimulus post-apnea would overcome the post-apneic sympathoinhibition. The lung inflation reflex is able to reduce the bradycardic response to neck suction of –30 Torr or less during inspiration; however, neck suction of –40 Torr or above overcomes the respiratory modulation of cardiac baroreflex responsiveness. Perhaps a similar phenomenon would occur post-apnea with the sympathetic arm of the carotid baroreflex.

III. To identify the mechanism of post-apneic sympathoinhibition, future experiments would likely have to move from human to animal research. The current studies demonstrate that the mechanism of post-apneic sympathoinhibition likely originates in the central nervous system, as peripheral reflexes do not appear to be the primary mediators. In addition, the immediacy of the sympathoinhibition upon apnea termination suggests a central nervous system mechanism. However, animal research would itself have many limitations. Animals cannot voluntarily control their respiration upon command as humans can; thus, apnea and its termination would have to be performed mechanically with the animal anesthetized. Additionally, to measure central neural pathways would also

require the animal to be anesthetized and surgery performed. These factors could confound the results and make interpretation difficult.