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This study was designed to test the hypothesis that an age-related vagal dysfunction compromises arterial blood pressure (ABP) regulation. Changes in heart rate (HR) and ABP during lower body negative pressure (LBNP) were compared between ten elderly (≥60 yrs) and ten young (≤30 yrs) adults. A separate, young group (n=10) was also assessed following muscarinic cholinergic (MC) blockade with atropine (central and peripheral receptor blockade) or glycopyrrolate (peripheral receptor blockade) to simulate vagal dysfunction. During the onset of LBNP –40 torr, orthostatic hypotension (OH) was observed in both the older subjects and the post-blockade younger subjects, with a diminished HR response. Furthermore, the reflex response to hypertensive stimuli was augmented in the post-blockade younger subjects, also associated with a diminution in HR response. We concluded that age-related or pharmacologically simulated vagal dysfunction compromises ABP regulation during hypotensive and hypertensive stimuli, and that the difference between atropine and glycopyrrolate was insignificant.

PERIPHERAL AND CENTRAL MUSCARINIC CHOLINERGIC RECEPTORS

IN ARTERIAL BLOOD PRESSURE REGULATION

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PERIPHERAL AND CENTRAL MUSCARINIC CHOLINERGIC RECEPTORS IN ARTERIAL BLOOD PRESSURE REGULATION

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LIST OF ABBREVIATIONS

ABP	Arterial Blood Pressure
ACh	Acetylcholine
CNS	Central Nervous System
CO	Cardiac Output
CVP	Central Venous Pressure
DBP	Diastolic Blood Pressure
FVR	Forearm Vascular Resistance
HF	High Frequency
HFNU	Normalized High Frequency
HR	Heart Rate
LBNP	Lower Body Negative Pressure
LF	Low Frequency
LFNU	Normalized Low Frequency
MAP	Mean Arterial Pressure
MC	Muscarinic Cholinergic
MSNA	Muscle Sympathetic Nerve Activity
NE	Norepinephrine
NTS	Nucleus Tractus Solitarius
OH	Orthostatic Hypotension
PE	Phenylephrine
PP	Pulse Pressure
PSA	Power Spectral Analysis
PSNA	Parasympathetic Nerve Activity
PVR	Peripheral Vascular Resistance
RRI	R-wave (pulse) Interval
SBP	Systolic Blood Pressure
SNA	Sympathetic Nerve Activity
SV	Stroke Volume

CHAPTER I

INTRODUCTION

During the activities of daily life, homeostatic mechanisms are constantly acting to maintain adequate arterial blood pressure (ABP). Receptors of the vasculature are particularly sensitive to changes in pressure that might compromise an adequate supply of blood to the tissues, and respond with multiple compensatory mechanisms to any significant deviations. The baroreceptor reflex is the first line of defense in response to changes in ABP. Changes in baroreceptor activity provide an error signal, activating several cardiovascular reflexes to reestablish blood pressure homeostasis. Without such a design, the pressure variations that accompany postural changes could never be withstood.

Cardiovascular reflexes are essential for maintaining ABP during postural changes. Gravity profoundly influences the circulation of blood, creating a hydrostatic column upon assumption of an upright posture that shifts approximately 70% of total blood volume to below the level of the heart (33). This translocation of blood to the compliant veins quickly lowers venous return and cardiac output, eliciting a hypotensive baroreceptor response. Gravitational stress creates a challenge for maintaining adequate cardiac output, since the venous circulation must successfully deliver a large quantity of blood back to the heart with no change in driving force.

The shift in blood volume that occurs during postural changes is normally compensated through baroreflex modulation of heart rate (HR), myocardial contractility, and vasomotor tone (37). As gravity translocates blood volume towards the feet, the expected decline in ABP is seen, creating a transient hypotension. By decreasing arterial pressure, baroreceptor stretch is reduced, and afferent nerve firing frequency declines. The reduced afferent nerve traffic has two important connotations for autonomic cardiovascular control. Parasympathetic innervation to the heart exists primarily at the nodal cells, where it can evoke rapid (1.0 – 1.5s) changes in heart rate (39). Transient hypotension decreases efferent vagal nerve activity, reducing the bradycardiac effect of vagal tone. This initial increase in heart rate is predominantly determined by cardiac vagal withdrawal (39). The progressive decline in thoracic blood volume upon orthostasis quickly reduces central venous pressure to near zero, reducing ventricular filling and stroke volume (33). While the immediate increase in HR temporarily improves cardiac output (CO), further compensation is needed to maintain ABP.

The sympathetic response to baroreceptor signaling is more delayed (5-10s) (33), involving many more interneuronal connections among nuclear regions than the parasympathetic pathway. Hypotensive stimuli reduce the tonic inhibition of sympathetic nerve activity (SNA) at the nucleus tractus solitarius (NTS), thus increasing adrenergic stimulation of heart rate. In addition, vasomotor tone is increased in an attempt to centralize blood volume and restore ABP (33). Enhanced SNA also promotes the release of renin from the juxtaglomerular cells to initiate the renin-angiotensin-aldosterone cascade, which promotes the increase in blood volume through a variety of mechanisms (13). The formation of angiotensin II is essential for proper vasoconstriction to counteract reductions in arterial pressure. These neurohumoral

compensations are thought to play a role in prolonged orthostatic stress.

This complex series of compensatory responses to the upright posture seems redundant, but are quite effective under normal circumstances. Young, healthy individuals respond quickly and effectively to orthostatic stress (39). Unfortunately, the elderly are less able to withstand the large changes in ABP that accompany postural change (9, 17, 38). Ineffective compensatory adjustments often reduce CO, limiting cerebral blood supply and creating conditions of dizziness and even pre-syncope in some individuals. Such symptoms are attributed to an age-related inability to adapt to hypotensive stimuli (24).

Several studies have clearly demonstrated a relationship between aging and the ability of the baroreflex to sense ABP changes and elicit such compensatory responses. It is well established that baroreflex responsiveness is attenuated with age (8, 18). Vagal modulation of HR in the elderly is significantly less responsive to hypertensive (18) and hypotensive (7, 19) stimuli when compared to young adults. Without the appropriate baroreflex mediated HR changes, adequate ABP may not be maintained upon orthostatic challenge.

Older individuals who are unable to maintain ABP upon standing (>20 mmHg drop in systolic blood pressure (SBP)) are clinically classified as having age-related orthostatic hypotension (OH) (43). A recent Cardiovascular Health Study survey found this condition was quite prevalent, affecting 17% (age 65-74) to 26% (>85 years of age) of the elderly population (34). Other studies claim the incidence of OH is as low as 7% (24) and as high as 30% (30) in healthy older people. Clearly, further investigation is needed to clarify the clinical picture and present potential methods of effective intervention.

While the clinical manifestations of this disorder are apparent, an explanation of the exact

mechanism has yet to be fully identified. Isolation of the location responsible for the reduced baroreceptor responsiveness must include consideration of the baroreceptor/afferent nerve(s), the end-organ receptors/efferent nerve(s), and the central integration of the baroreceptor signal at the medullary cardiovascular centers. Many physiological studies have been designed to clarify each segment of the baroreflex pathway.

It is well established that arterial compliance decreases with age (16), related to a progressive increase in the collagen/elastin ratio. Baroreceptor axons terminate in the vascular wall, in close approximation to the deformable elements of the vessel (elastic fibers, smooth muscle cells, collagen bundles) (32). In addition to imposing a greater afterload to the ventricles, it is easy to imagine how diminished vascular compliance could potentially alter baroreceptor sensitivity.

Several studies have revealed that many receptor types undergo functional changes with advancing age. Beta (β) adrenoreceptor quantity remains unchanged (2, 12) or decreases (14, 23), and affinity for receptors is reduced (15). More recent receptor studies suggest a decline in function of the muscarinic cholinergic (MC) receptors on the myocyte (22, 31). Biological age does not appear to affect alpha (α) adrenoreceptors (28, 41), though recent studies imply an age-related decline in α-1 receptor responsiveness may exist (10). Clearly, diminished receptor responsiveness could contribute to the age-related decrease in baroreflex activity. Docherty was indeed understated in noting the difficulty of separating the neural reflex effect from the diminished capability of the end organ (11).

Studies investigating the age-related changes in central baroreflex control offer few definitive conclusions. Descriptions of the specific nuclear regions, types of neurotransmitters and

receptors, and interneuronal connections involved are limited. Since central integration involves both autonomic efferent branches, the interaction is further complicated. As a result, investigators have worked to devise techniques for analyzing the individual components of the cardiovascular center.

Traditionally, forearm vascular resistance (FVR), muscle sympathetic nerve activity (MSNA), and plasma norepinephrine (NE) are taken as a measure of SNA (36). Based on these variables, studies have often concluded that sympatho-circulatory control is attenuated with age (20, 40). However, more recent studies suggest that arterial baroreflex control of sympathetic nerve activity is preserved with age. Matsukawa *et al* measured MSNA during pressor and depressor responses to determine how the baroreflex control of SNA is affected by aging (25). They found baroreflex control of SNA to be preserved despite attenuation of parasympathetic nerve activity (PSNA). In addition, Davy *et al* recently reported that baroreflex control of sympathetic outflow during hypovolemia, measured by MSNA, is augmented with age (10). In this study, the authors attribute the attenuated FVR response to a non-neuronal change in vasoconstrictor responsiveness. These and other studies hint at the complexity of autonomic balance in ABP control.

Power spectral analysis of HR and ABP are gaining attention as a means of quantifying sympatho-vagal balance in cardiovascular control (29). Beat-to-beat oscillations in HR and ABP were once deemed measurement "noise", but these rhythms are now considered as a means of quantifying cardiovascular control (3). The variability in HR and ABP signals can thus be evaluated through spectral analysis techniques to provide an index of autonomic function (21). This technique has the potential to further clarify the central nervous system (CNS) integration of

baroreflex signaling. An appendix describing the use of power spectral analysis in this study can be found in chapter II, manuscript two.

Measurements of HR and ABP variability after pharmacologic blockade of specific receptors have provided a wealth of information concerning sympatho-vagal balance under various conditions (25). Recently, the interaction among the autonomic branches was further considered in a study using atropine for central MC receptor blockade (26). Since central muscarinic effects on HR are masked by blockade at the sinoatrial (SA) node, MSNA was used as an index of SNA. Power spectral analysis was employed to analyze MSNA variability in both low (LF) and high frequency (HF). Atropine administration produced a significant reduction in both MSNA burst frequency and LF_{MSNA} variability, with an increase in SBP and HF_{MSNA}. Interestingly, the authors suggested that atropine-induced central parasympathetic *activation* modulated sympathetic nerve traffic to peripheral vessels.

HR and ABP variability also prove useful in evaluating cardiovascular control during dynamic conditions, such as orthostatic stress (27). In 1997, Hayes *et al* (19) identified a greater decrease in mean arterial pressure (MAP) associated with a diminished tachycardia at the onset of lower body negative pressure (LBNP) in the elderly compared to young adults. This attenuated tachycardiac reflex at the onset of orthostatic challenge results primarily from an age-related change in vagal control of HR, as indicated by the diminished HF power spectrum of HR variability (42). However, ABP is typically maintained within one minute of sustained orthostatic challenge, suggesting a vasoconstrictory compensation to maintain normal ABP (35). These studies indicate an age-related deterioration of blood pressure regulation in the short-term response to orthostatic challenge, which may be of serious consequence to the elderly population.

The strategy of the current study was to combine pharmacologic blockade with orthostatic stress, and evaluate the changes in ABP regulation. In aging studies, it is often difficult to differentiate vagal dysfunction from other age-related disorders that have varying effects on the autonomic nervous system. Cholinergic blockade simply *mimics* the age-related changes in baroreflex function, thus eliminating other age-related alterations in cardiovascular function. Our study employed only young, healthy subjects, and performed pharmacological simulation of vagal dysfunction in an attempt to eliminate confounding variables. Atropine was used to eliminate the age-related difference in HR changes mediated by vagal influence, non-selectively blocking both central and peripheral MC receptors. Since glycopyrrolate influences only peripheral MC receptors, comparison of the two drugs may further elucidate the central integration of baroreflex signaling. Such information has the potential to assist clinicians in treatment of orthostatic hypotension associated with autonomic dysfunction.

Summary: The purpose of this study is to compare ABP regulation in LBNP-induced central hypovolemia following MC receptor blockade. Atropine and glycopyrrolate are anticholinergic drugs that eliminate vagal modulation, thus mimicking age-related vagal dysfunction. Atropine is known to penetrate the blood brain barrier, blocking both central and peripheral MC receptors (1). Glycopyrrolate is also an MC antagonist, but has little influence on the CNS (5). The potency of the two drugs on HR is comparable in humans when effective doses are administered to establish MC receptor blockade (4, 6). Use of these MC antagonists will allow us to better characterize the role of central and peripheral MC receptors in ABP regulation.

Research Objectives:

The first objective of the study is to further establish the importance of vagal modulation in regulation of ABP. Simulated orthostatic stress before and after pharmacologic abolition of vagal influence will reveal its significance in maintaining ABP. If the loss of vagal modulation from peripheral MC receptor blockade does in fact alter ABP regulation, will addition of a central blockade create further dysfunction? The second objective is to distinguish the effect of the central from the peripheral MC receptors on ABP regulation by comparing the cardiovascular reflex responses following MC receptor blockade using atropine or glycopyrrolate. Thirdly, the experiment evaluates ABP regulation in response to both onset and sustained central hypovolemic challenge using moderate to maximal LBNP. A graded LBNP test will be performed before and after drug injection, as we believe vagal blockade will alter the function of ABP regulation during simulated orthostatic stress.

Specific Research Questions:

Question one: In young adult subjects, do both atropine and glycopyrrolate successfully simulate the diminished HR reflex observed in the elderly population?

The vagus nerve innervates the sinus pacemaker, releasing the neurotransmitter acetylcholine (ACh). The MC receptors on the nodal cells bind this ACh, increasing the time required for the action potential to reach threshold. Both atropine and glycopyrrolate occupy these MC receptors, effectively eliminating the vagal influence on the heart. By comparing the HR reflex of the young group with that of the elderly, we hope to provide evidence to support the use of vagal blockade to simulate age-related vagal dysfunction.

Question two: Is there a difference between central and peripheral MC receptors in regulation of ABP and reflex control of heart rate?

Comparison of atropine and glycopyrrolate will differentiate the role of central and peripheral MC receptors in blood pressure regulation. Atropine is able to cross the blood-brain barrier and occupy central MC receptors in addition to blocking peripheral MC receptors of the sinoatrial (SA) node. By assessing ABP regulation both *i*) before and after each drug administration and *ii*) between the two drugs, we expect to establish the role of central MC receptors in vagal modulation. We hypothesize *both* central and peripheral MC antagonists will compromise the function of ABP regulation, and with the complexity of afferent signal integration, we expect that atropine blockade will differ from glycopyrrolate blockade.

Question three: Is there a different response to the onset of LBNP or sustained LBNP after either atropine or glycopyrrolate?

Beat-to-beat data for baseline, onset, and sustained LBNP before and after injection of either atropine or glycopyrrolate will be collected. During LBNP simulated orthostasis, the hypotensive "error signal" is disrupted both centrally (atropine) and peripherally (atropine and glycopyrrolate). Analysis of the changes in HR and ABP during these various conditions will provide additional information regarding vagal dysfunction.

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CHAPTER TWO

MANUSCRIPTS

The following manuscripts contain data from two separate studies. Patrick M. Hayes, Hong-Wei Wang, and Xiangrong Shi conducted study number one in 1997, and the results have yet to be published in manuscript form. Study number two was conducted by David Walter Wray, Kevin J. Formes, and Xiangrong Shi in the summer of 1998. The two studies addressed similar issues, but used two markedly different approaches. Upon completion of the second study, it was realized that some of the data would be best expressed in conjunction with the first study. Thus, two separate manuscripts were produced to address the issues of arterial blood pressure regulation and age.

Study number one, Aging and Orthostatic Hypotension, used lower body negative pressure (LNBP) to simulate orthostasis in young and older individuals. In the elderly, an orthostatic hypotension was observed during the first few seconds (onset) of orthostatic stress due to a lack of reflex tachycardia, attributed to a dimunition of vagal function. However, there was no age-related difference in arterial pressure changes after one minute of LBNP, suggesting that a vasomotor response was responsible for arterial blood pressure regulation during sustained orthostatic stress. This vasoconstrictor response appeared to compensate for the diminished reflex tachycardia experienced during LBNP.

Study number two, Importance of Vagal-Cardiac Influence in Arterial Blood Pressure Regulation, applied muscarinic cholinergic (MC) antagonists to *simulate* this age-related vagal dysfunction in young individuals. This technique resulted in a similar loss of vagal function, and the cardiovascular response to LBNP was identical to the elderly group. Thus, we chose to include the LBNP onset response of these young individuals in study number one as further evidence of vagal dysfunction as the cause of orthostatic hypotension in the elderly. Study two also investigated the differences between peripheral and central MC receptors to further emphasize the importance of vagal function in the maintenance of arterial blood pressure homeostasis.

ORTHOSTATIC HYPOTENSION IN AGING HUMANS

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ABSTRACT

This study was designed to test the hypothesis that healthy older adults show a greater hypotension at the onset of orthostatic challenge, and that this response is due to an age-related *vagal dysfunction.* Responses of pulse interval (RRI), arterial blood pressure (monitored by intra-arterial catheter or Finapres), and central venous pressure (CVP) were compared between ten healthy older (≥60 yr-old) and ten younger (<30 yr-old) adults during onset (the first 10 pulses) and sustained lower body negative pressure (LBNP). A younger group was also assessed following full blockade of vagal influence using atropine or glycopyrrolate. Baseline RRI, CVP, and mean arterial pressure (MAP) were similar between the groups. LBNP of -15 torr significantly decreased CVP without hypotension in both groups. During -40 torr, a decrease in RRI occurred at the first pulse wave in the younger subjects without hypotension. However, an orthostatic hypotension was observed in the older subjects without tachycardia. Following atropine or glycopyrrolate, tachycardiac responses of younger subjects were significantly diminished, and were associated with a systemic hypotension at the onset of LBNP -40 torr. However, this age-related or drug-induced orthostatic hypotension was minimal during sustained LBNP. We concluded that older adults experience orthostatic hypotension at the onset of LBNP -40 torr due to a diminished tachycardia caused by *vagal dysfunction.* However, a compensatory augmentation of vasoconstriction enables them to maintain arterial blood pressure during sustained LBNP if the vasomotor response is not compromised.

Key Words: *aging, orthostatic hypotension, central hypovolemia, reflex tachycardia, lower body negative pressure, atropine, glycopyrrolate*

INTRODUCTION

Orthostatic hypotension, defined as a decrease in systolic blood pressure (SBP) of ≥20 mmHg or in diastolic blood pressure (DBP) of ≥10 mmHg, is prevalent with age (17). It has been reported that up to 30% of normotensive subjects over 65 years of age experience a decrease in SBP ≥20 mmHg during 60° head-up tilt (16). However, the incidence of orthostatic hypotension (OH) observed in the elderly population is frequently complicated by age-related pathological conditions, such as high blood pressure (5, 10), or by medications for these conditions, such as antihypertensive agents (12, 24). Arterial blood pressure (ABP) regulation during orthostatic challenge, elicited by standing or simulated by lower body negative pressure (LBNP), appears to be functional in healthy, normotensive older adults compared to their younger counterparts (23). Though arterial baroreflex control of heart rate (HR) is significantly diminished with age, muscle sympathetic nerve activity (MSNA) (7) and venous plasma norepinephrine (NE) concentration (23) during hypotensive stimuli are not different between younger and older adults. Since the neurally and humorally mediated vasomotor responses take longer to be effective, it remains questionable whether elderly people exhibit a greater hypotension at the onset of orthostatic challenge compared to their younger counterparts. We postulated that age compromises the rapid response of ABP regulation at the onset of orthostatic challenge because of an impaired tachycardiac response caused by an age-related vagal dysfunction. The purpose of this study was to determine whether OH was present in older adults at the onset of LBNP-induced central hypovolemia because of an age-related decrease in reflex tachycardia. If the diminution of the vagal function was the mechanism responsible for the orthostatic hypotension in older adults, then this aging phenomenon could be imitated in younger

subjects following administration of a muscarinic cholinergic (MC) antagonist to block the parasympathetic influence, thus mimicking the age-related vagal dysfunction. Since the MC antagonist atropine penetrates the blood-brain barrier and blocks both central and peripheral MC receptors (13), we also chose the MC antagonist glycopyrrolate, which presumably remains in the periphery (2, 3). Use of these two drugs would allow differentiation of central modulation on ABP regulation.

METHODS

Subjects. Ten (5 men and 5 women) younger subjects (25±1 yrs) and ten (5 men and 5 women) older subjects (64±1 yrs) participated in the first study. All younger and older subjects were normotensive, without a medical history, and taking no medications during the study. Body weight (71.1±4.9 and 74.0±5.0 kg) and height (173±3 and 171±3 cm) were similar between the younger and older subjects. The second study tested 10 (7 men and 3 women) healthy younger adults (24±1 yrs) with and without the muscarinic cholinergic (MC) antagonists atropine and glycopyrrolate. After passing a physical examination, all subjects signed an informed consent, which explained the purpose and procedure of the experiments. The experimental procedure and the consent form were approved by the Institutional Review Board of the University of North Texas Health Science Center at Fort Worth.

Protocol. Before the test, each subject was oriented to the laboratory and familiarized with the experimental procedure and measurements to be used during the test. All experiments were carried out with the subjects lying supine with the lower body in a LBNP box.

Study 1: After sealing the box, negative pressure was pre-set at -15 torr. The subject's body was prevented from moving during LBNP by a cushioned saddle inside the box between the subject's legs. Following ≥30 min of supine rest, baseline pulse interval (RRI), heart rate (HR), systolic, diastolic, and mean arterial pressure (SBP, DBP, and MAP), and central venous pressure (CVP) was beat-to-beat recorded by an on-line computer. Immediately following 1-min baseline, negative pressure was established and maintained for 8-10 min. Cardiovascular variables were continuously monitored during LBNP. After ≥10 min of recovery from LBNP -15 torr, baseline data was again collected, followed by LBNP of -40 torr for 8-10 min.

Study 2: The second group of younger subjects performed two LBNP tests at -40 torr on each experimental day. Baseline HR and arterial blood pressure (ABP) were continuously collected for 1-min, followed by the application of LBNP, which was pre-set at -40 torr. After recovery from LBNP, atropine (n=8) was injected at 5 μg/kg to fully block the muscarinic cholinergic (MC) receptors, i.e., there was no further tachycardia observed after two consecutive doses, or to a cumulative dose of 40 μg/kg body weight. After one week, subjects returned to the lab to repeat the same protocol with glycopyrrolate (n=8) as the MC antagonist. Glycopyrrolate was injected at 2 μg/kg until complete blockade was achieved, or to a cumulative dose of 16 μg/kg. The doses of glycopyrrolate and atropine were equi-potent (12-14). The test order was randomized.

Measurements. All experiments were conducted with an ambient temperature between 24-26°C and relative humidity 55% - 65%. A standard II lead electrocardiogram was used to monitor HR.

For study 1, ABP was continuously measured by an intra-radial arterial catheter (9 younger and 2 older subjects) or by a finger cuff (Finapres, Ohmeda) on the middle finger. CVP was determined (in 6 younger and 2 older subjects) by a double lumen catheter (Cook Critical Care) inserted through the right basilic vein. The tip of the catheter was advanced to between the 3rd and 4th intercostal space under the supervision of fluoroscopy (BV22, Philips, Eindhoven, Netherlands). For study 2, ABP was directly monitored in the first test, and radial tonometry (Colin 7000) was used for test 2. Both ABP and CVP were interfaced with sterile disposable pressure transducers (Cobe, Lakewood, CO) and monitored by a dual-pressure channel monitor (Hewlett-Packard 78342A). Zero point of both pressure transducers was leveled at the subject's mid-axillary line. During all experiments, the pressure inside the LBNP box was continuously monitored.

Data Management. Data were reported in group means ± the standard error of the means. Changes in RRI, SBP, DBP, MAP, and CVP during the initial 10 pulses and during the 1st, 2nd, 3rd, and 8th min of LBNP were calculated as the cardiovascular responses to the onset of and sustained orthostatic stresses, respectively. Two-way analysis of variance (ANOVA) was employed to determine the age and time (during LBNP) factors (in study 1), or the effects of MC antagonists and time in the younger group (in study 2), on these cardiovascular responses. Duncan's method was used to compare the difference of the first 10 pulse responses at the onset of LBNP. Tukey's methods were applied for post-hoc analysis during LBNP, if ANOVA outcome was significant for the time (min) factor. Statistic Analysis System (SAS) software was utilized for the significance analysis. A P value of ≤ 0.05 was considered significant.

RESULTS

Study 1: All subjects' arterial blood pressures (ABP) were within normotensive range. Mean and diastolic arterial pressures (MAP, DBP) tended to be higher in the older group, whereas central venous pressure (CVP) tended to be higher in the younger group (see tables 1 and 2). However, none of these differences reached a $P \leq 0.05$ level. Heart rate (HR) and pulse interval (RRI) values were statistically identical in the younger and older subjects.

LBNP -15 and -40 torr produced a significant central hypovolemia (indicated by a decrease in CVP) in both younger and older subjects (tables 1 and 2). Figure 1 illustrates that changes in CVP in a younger and an older subject faithfully follow the application of LBNP at -15 and -40 torr from initiation to the pre-set LBNP level. Average time from the initiation of LBNP to the steady state was 5.8±0.2 sec. This transition time was not different between -15 and -40 torr LBNP in either younger (5.9±0.5 and 5.4±0.4 sec) or older (6.1±0.4 and 5.9±0.3 sec) subjects.

ABP during LBNP -15 torr was well maintained in both age groups (figure 2 and table 1). Baseline cardiovascular data prior to LBNP -15 and -40 torr were not statistically different in either age group. When LBNP -40 torr was applied, a significant systemic hypotension accompanied by an absence of tachycardia within the first 10 pulses was observed in the older subjects (figure 3 and table 2). In contrast, the younger group experienced a significant tachycardiac response at the onset of LBNP without hypotension. However, the age-related difference in the change of ABP was absent after 1 min LBNP -40 torr, despite significantly decreased tachycardia in the older subjects. The decrease in CVP during LBNP tended to be greater in the younger group than the older group (table 2).

Study 2: Baseline RRI, ABP, and their responses to LBNP -40 torr before atropine and glycopyrrolate were not statistically different. Therefore, these baseline data were merged into one "*control*" group. Muscarinic cholinergic (MC) antagonists increased HR ($P<0.001$), but did not significantly affect ABP (table 3). The effect between drugs was not significantly different. During the control condition (i.e., before drug), ABP was well maintained during LBNP -40 torr, with a significant tachycardiac response (figure 4 and table 3). However, following atropine or glycopyrrolate to block vagal influence, a systemic hypotension occurred, associated with a significantly diminished tachycardiac response at the onset of LBNP. This response was similar to that observed in the older adults (figure 3). The changes in RRI and ABP were similar between atropine and glycopyrrolate. During sustained LBNP, the difference among the experimental conditions was insignificant.

DISCUSSION

The major finding of this study confirms that a significant hypotension in normal, healthy older adults, but not in the younger counterparts, occurs at the onset of lower body negative pressure (LBNP) induced orthostatic challenge, suggesting that aging without complication of diseases diminishes the immediate response of arterial blood pressure (ABP) regulation. The underlying mechanism appears to be an age-related *vagal dysfunction*, since a similar systemic hypotension occurs in the younger subjects following administration of the muscarinic cholinergic (MC) antagonist atropine or glycopyrrolate. However, the initial orthostatic hypotension (OH) is not present during sustained LBNP. Our data also suggest that LBNP -15 torr does not significantly decrease ABP in either the younger or older groups.

The present investigation demonstrated that the OH observed in older adults at the onset of LBNP -40 torr (figure 3) was related to a diminished baroreflex control of heart rate (HR). This OH cannot be attributed to a loss of vasomotor responsiveness in the elderly, since the reflex response in muscle sympathetic nerve activity (MSNA) (7) and venous plasma norepinephrine (NE) concentration (23) during hypotensive stimuli appear unaffected by age. Consequently, the increases in peripheral vascular resistance (PVR) during steady-state LBNP are thought to be similar between healthy older and younger subjects (18). Recent data implied that a reflex increase in forearm vascular resistance was less in older subjects in terms of the unit increase in MSNA (6), most likely due to a desensitization of α-adrenoceptors. However, an age-related difference in the vasomotor response could not be responsible for the *initial* OH observed in the older subjects, since the contribution of vasomotor tone to blood pressure regulation takes longer to be effective. Our data indicate that reflex tachycardia plays a crucial role in the maintenance of ABP at the onset of orthostatic challenge.

Reflex tachycardia can be caused by vagal withdrawal or sympathetic activation (14, 19-22). The present data confirm that vagal withdrawal is the dominant factor for the rapid tachycardiac response to maintain hemodynamic homeostasis at the onset of orthostatic stress. Recent observations in our lab (9) demonstrated that the tachycardiac response to the onset of LBNP –40 torr was not different before and after selective cardiac sympathetic blockade with metaprolol. However, following MC antagonists in the younger adults, the tachycardiac response to the onset of LBNP was significantly diminished, associated with a significant systemic hypotension (figure 4 and table 3). However, the difference in systemic hypotension before and after MC receptor antagonists was not significant during sustained LBNP. The increase in HR

(in terms of beats/minute) after MC receptor antagonist administration tended to be greater after 1-min LBNP, suggesting a slow and augmented cardiac sympathetic activation. The difference in hemodynamic responses observed between atropine and glycopyrrolate was insignificant, though it has been noticed that low doses of atropine decelerate heart rate (13) as a result of central interference (8).

Though a systemic hypotension was present in the older group at the onset of LBNP -40 torr and a diminished tachycardiac response persisted in the older subjects during sustained LBNP -40 torr, there was no age-related difference in the change of ABP pressure after 1-min LBNP. These data suggest that a neurohumorally mediated vasomotor response predominates ABP regulation during sustained orthostatic challenge, and that this vasomotor mechanism compensates for the age-related diminution of reflex tachycardia in the elderly. Our data indicated that an initial OH could be corrected by the reflex vasomotor response in older adults if they are free from hypertension or any other disease secondary to aging. Without the vasoconstrictor compensation, however, the initial OH could lead to orthostatic intolerance or syncope.

LBNP significantly reduced central venous pressure (CVP) in both the younger and older subject groups. Therefore, a greater reduction of ABP in the older subjects during LBNP -40 torr could not be attributed to a difference in the reduction of venous return between the groups. In fact, the decrease in CVP tended to be greater in the younger than in the older subjects (Table 2). This result appears to be consistent with a recent observation that peripheral venous compliance, as indicated by an increase in leg volume per unit increase in leg muscle interstitial pressure elicited by LBNP, is significantly decreased in older adults (15). However, CVP is not only

determined by the venous return from the periphery, but also affected by the cardiac pump capacity. An age-related decrease in peripheral venous compliance (15) may impede venous pooling, so that the reduction of CVP is less during LBNP. An augmented cardiac inotropic or chronotropic function, which pumps a greater amount of the cardiopulmonary volume into the periphery, may also decrease CVP. This could provide an alternate explanation for a greater reduction of CVP observed in the younger subjects.

Though a significant decrease in CVP was observed during LBNP -15 torr, there was no significant hypotension in either the younger or the older group. This suggests that a vasoconstrictor response completely compensated for the LBNP-induced central hypovolemia, and that the vasoconstriction was predominantly mediated by the cardiopulmonary baroreflex. These data are consistent with findings from previous studies (1, 11, 26). It is generally believed that LBNP beyond -20 torr is able to unload both cardiopulmonary and arterial baroreceptors (1, 11, 26), and that LBNP -50 torr elicited orthostatic stress is similar to passive standing or head-up tilt +70° assessed by the changes in HR and ABP (4, 25).

In summary, the present investigation indicates that healthy older adults may experience OH during body postural transitions to the upright position, as seen at the onset of LBNP -40 torr. The underlying mechanism is an age-related diminution of reflex tachycardia due to *vagal dysfunction*. However, an augmented vasoconstrictor response is able to compensate for the diminished reflex tachycardia and maintain ABP during a steady state orthostatic challenge, if human aging is not complicated by disease. We concluded that OH did occur in normal, healthy older adults during orthostatic challenge, but that an augmented vasomotor response prevented orthostatic intolerance or syncope in these individuals.

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FIGURE LEGEND

Figure 1: Representative central venous pressure (CVP, solid line) from a younger subject and a older subject during supine rest and lower body negative pressure (LBNP, dotted line) -15 and -40 torr.

Figure 2: Responses of pulse interval (ΔRRI) and systolic arterial pressure (ΔSBP) within the first 10 pulses at the onset of LBNP and during min 1, min 2, min 3, and min 8 of -15 torr LBNP. Baseline RRI and SBP prior to-15 torr LBNP are similar between the younger (n=8, 1048±77 ms and 123±4 mmHg) and older (n=9, 1118±37 ms and 124±5 mmHg) subjects.

Figure 3: Responses of pulse interval (ΔRRI) and systolic arterial pressure (ΔSBP) within the first 10 pulses at the onset of LBNP and during min 1, min 2, min 3, and min 8 of -40 torr LBNP. * and # denote a significant change from the baseline and baseline plus min 1 data, respectively. Baseline RRI and SBP prior to-40 torr LBNP are similar between the younger (n=10, 1081±58

ms and 122±4 mmHg) and older (n=10, 1112±35 ms and 129±6 mmHg) subjects. Orthostatic hypotension (OH) is observed in the older subjects at the onset of LBNP only. The ΔSBP was not different between the groups from minute 2 LBNP, though tachycardia is still less in the older subjects.

Figure 4: Responses of pulse interval (ΔRRI) and systolic arterial pressure (ΔSBP) of younger subjects at the onset of LBNP and during min 1, min 2, min 3, and min 8 of –40 torr LBNP with and without muscarinic cholinergic antagonists. * denotes a significant change from the baseline. RRI is significantly decreased (from 1028±51 ms) to 604±22 and 567±19 ms following atropine and glycopyrrolate blockade. However, baseline SBP was not significantly affected by drugs (control: 123±3 mmHg, n=10; atropine: 126±3 mmHg, n=8; glycopyrrolate: 128±5 mmHg, n=8). A significant systemic hypotension is observed in the younger subjects following vagal blockade using either atropine or glycopyrrolate, which is associated with a substantially blunted tachycardiac response at the onset of LBNP. During sustained LBNP, the difference among the three conditions is not significant.

Table 1 : Cardiovascular responses during LBNP –15 Torr.

Variable	Group	Base	ΔP1	ΔP2	ΔP3	ΔP4	ΔP5	ΔP6	ΔP7	ΔP8	ΔP9	ΔP10	ΔM1	ΔM2	ΔM3	ΔM8
MAP (mmHg)	Young	83±2	+3±2	+3±2	+3±2	+1±2	0±2	-1±2	-1±2	-2±2	-3±2	-3±1	-1±1	0±1	0±1	-2±1
	Old	89±3	-3±2	-2±2	-3±2	-3±2	-3±2	-4±2	-5±2	-5±2	-4±2	-4±2	-2±2	-2±2	-2±2	-4±3
	P	0.190	0.071	0.046	0.030	0.079	0.206	0.244	0.235	0.453	0.799	0.507	0.560	0.500	0.458	0.607
DBP (mmHg)	Young	65±1	+3±2	+3±2	+3±2	+2±1	+1±1	0±2	-1±2	-2±2	-2±1	-2±1	0±1	+1±1	+1±1	0±1
	Old	68±2	-3±2	-2±1	-3±1	-3±1	-2±1	-3±2	-4±2	-3±2	-4±2	-2±2	-1±1	0±1	-1±2	-3±3
	P	0.361	0.032	0.021	0.014	0.060	0.189	0.231	0.252	0.482	0.402	0.638	0.551	0.483	0.451	0.387
CVP (mmHg)	Young	6.6±0.8	-4.2±0.7*	4.4±1.2*	-3.6±1.2*	-5.5±2.5*	5.5±2.3*	-6.0±1.9*	-4.6±1.3*	-4.7±1.0*	-4.9±1.9*	-7.4±2.6*	-5.9±1.6*	-5.5±1.2*	-5.2±1.1*	-4.8±1.6*
	Old	4.2±0.4	-2.2±0.1*	-2.8±1.3*	-1.5±0.6*	-3.1±1.0*	-3.0±0.3	-3.1±0.8*	-1.8±0.3	-2.3±0.1*	-2.6±0.4*	-3.5±1.2*	-2.6±0.3*	-2.3±0.2*	-2.1±0.1*	-1.8±0.5*
	P	0.122	0.091	0.458	0.322	0.547	0.514	0.383	0.211	0.195	0.469	0.379	0.249	0.147	0.124	0.293
HR (bpm)	Young	59±4	+3±3	+4±3	+5±3	+6±3	+6±3	+7±2	+6±2	+6±2	+6±2	+6±2	+4±2	+5±2	+4±1	+6±2
	Old	54±1	+2±1	+2±1	+2±1	+3±1	+3±1	+3±1	+2±1	+3±1	+2±1	+2±1	+2±1	+3±1	+3±1	+3±1
	P	0.258	0.532	0.406	0.308	0.231	0.166	0.140	0.091	0.130	0.141	0.082	0.171	0.172	0.243	0.038

Base represents baseline data prior to LBNP. ΔP1 - ΔP10 are the responses of the 1st to the 10th pulses during LBNP -15 torr.

ΔM1 - ΔM8 are the data averaged from min 1, min 2, min 3 and min 8 during LBNP. n=8 in the younger group and n=9 in the older group in all variables, except in CVP: n=4 younger and n=2 older subjects. P value is the outcome of ANOVA for the difference between the groups. * indicates a significant change from the baseline. MAP and DBP: mean and diastolic arterial pressure; CVP: central venous pressure; HR: heart rate.

Table 2 : Cardiovascular responses during LBNP –40Torr.

Variable	Group	Base	ΔP1	ΔP2	ΔP3	ΔP4	ΔP5	ΔP6	ΔP7	ΔP8	ΔP9	ΔP10	ΔM1	ΔM2	ΔM3	ΔM8
MAP (mmHg)	Young	83±2	+1±1	-1±1	-3±1	-3±1	-3±1	-4±2	-5±2	-6±2	-5±2	-4±1	-2±1	-2±1	-2±1	-2±1
	Old	92±5	-7±2*	-10±3*	-13±4*	-13±4*	-15±4*	-16±4*	-15±4*	-14±4*	-14±3*	-12±3*	-6±2	-6±3	-7±4	-1±2
	P	0.190	0.002	0.006	0.010	0.017	0.007	0.010	0.026	0.036	0.029	0.038	0.057	0.157	0.229	0.656
DBP (mmHg)	Young	65±2	0±1	-1±1	-2±1	-2±1	-3±1	-3±1	-3±1	-4±1	-5±1	-3±1	+1±1	+2±1	+2±1	+2±1
	Old	71±4	-7±2*	-8±2*	-10±2	-8±3	-9±3*	-10±2*	-10±2*	-10±3*	-9±2*	-8±2*	-2±1	-1±2	-2±3	-3±3
	P	0.188	0.003	0.003	0.003	0.076	0.068	0.019	0.028	0.083	0.085	0.082	0.141	0.205	0.239	0.733
CVP (mmHg)	Young	7.4±0.8	-6.3±1.5*	-5.7±1.0*	-5.5±0.7*	-5.0±0.7*	-5.7±0.9*	-6.8±1.3*	-6.9±1.3*	-6.8±1.1*	-6.2±0.7*	-6.1±0.6*	-7.5±0.8*	-7.9±0.9*	-8.0±0.8*	-7.9±1.1*
	Old	3.9±0.9	-3.8±0.8*	-1.5±0.3*	-3.3±0.7*	-3.2±0.3*	-3.2±0.1*	-5.0±0.4*	-2.4±0.9*	-3.2±0.4*	-2.9±0.1*	-4.9±0.1*	-3.6±0.1*	-3.4±0.3*	-3.6±0.2*	—
	P	0.062	0.382	0.061	0.325	0.219	0.172	0.481	0.118	0.111	0.038	0.262	0.062	0.026	0.024	—
HR (bpm)	Young	57±3	+8±2*	+9±2*	+10±2*	+10±2*	+11±2*	+11±2*	+12±3*	+12±3*	+13±3*	+13±3*	+11±3*	+15±3*	+17±3*	+18±2*
	Old	55±2	+2±1	+3±1	+3±1	+3±1	+3±1	+3±1	+3±1	+3±1	+3±1	+3±1	+4±1	+7±1	+7±1	+9±2
	P	0.491	0.030	0.025	0.017	0.015	0.009	0.007	0.007	0.006	0.005	0.005	0.017	0.017	0.007	0.005

n=10 in both age groups, except in CVP: *n*=6 younger and *n*=2 older subjects. * indicates a significant change compared to the baseline plus the first min during LBNP. Baseline data prior to LBNP -15 and -40 torr are not different in either younger or older group.

Table 3: Cardiovascular responses of younger subjects during LBNP -40 torr with and without muscarinic cholinergic antagonists.

Variable	Group	Base	ΔP1	ΔP2	ΔP3	ΔP4	ΔP5	ΔP6	ΔP7	ΔP8	ΔP9	ΔP10	ΔM1	ΔM2	ΔM3	ΔM8
MAP MmHg	C	86±2	+2±2	+2±2	+3±2	+2±2	+1±2	0±2	+1±2	0±2	+1±2	0±2	0±1	-1±1	-2±1	-3±1
	A	88±3	-8±2*	-9±2*	-10±3*	-11±3*	-10±3*	-11±3*	-12±3*	-13±3*	-13±3*	-12±3*	-5±2	-3±2	-4±2	-4±2
	G	94±5	-10±1*	-11±2*	-12±2*	-13±2*	-14±3*	-15±3*	-16±3*	-16±4*	-15±5*	-15±5*	-5±4	-2±4	-1±3	-4±5
	P	0.164	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.003	0.006	0.299	0.884	0.661	0.990
DBP MmHg	C	67±1	1±1	+2±1	+2±1	+1±1	+1±2	+1±2	0±2	+1±2	0±2	+1±2	0±1	0±1	0±1	-2±1
	A	71±2	-8±3*	-10±3*	-11±3*	-11±3*	-11±3*	-11±4*	-13±3*	-14±3*	-13±3*	-13±3*	-4±2	-1±2	-2±2	-2±2
	G	76±5	-9±2*	-10±2*	-11±2*	-13±2*	-14±3*	-15±3*	-16±4*	-16±4*	-15±4*	-14±5*	-3±5	0±4	+1±4	-2±5
	P	0.111	0.002	0.001	0.001	0.001	0.001	0.001	0.002	0.002	0.005	0.006	0.484	0.899	0.727	0.992
HR Bpm	C	60±3	+5±3	+6±2*	+7±2*	+8±2*	+8±2*	+8±2*	+8±2*	+8±2*	+8±2*	+8±2*	+3±1	+2±1	+3±1	+4±1
	A	100±4	+1±1	+2±1	+3±1	+4±1	+4±1	+4±1	+5±1*	+5±1*	+5±1*	+5±1*	+7±1*	+8±2*	+9±2*	+8±2*
	G	107±4	-4±4	-4±4	-5±4	-1±3	0±2	0±2	+1±2	+1±2	+1±2	+4±1	+6±2	+9±2*	+8±2	+9±3*
	P	0.001	0.044	0.027	0.017	0.031	0.018	0.021	0.046	0.057	0.091	.0218	.0167	0.014	0.048	.0289

C: before drug (n=10); A: atropine (n=8); G: glycopyrrolate (n=8).

Figure 1

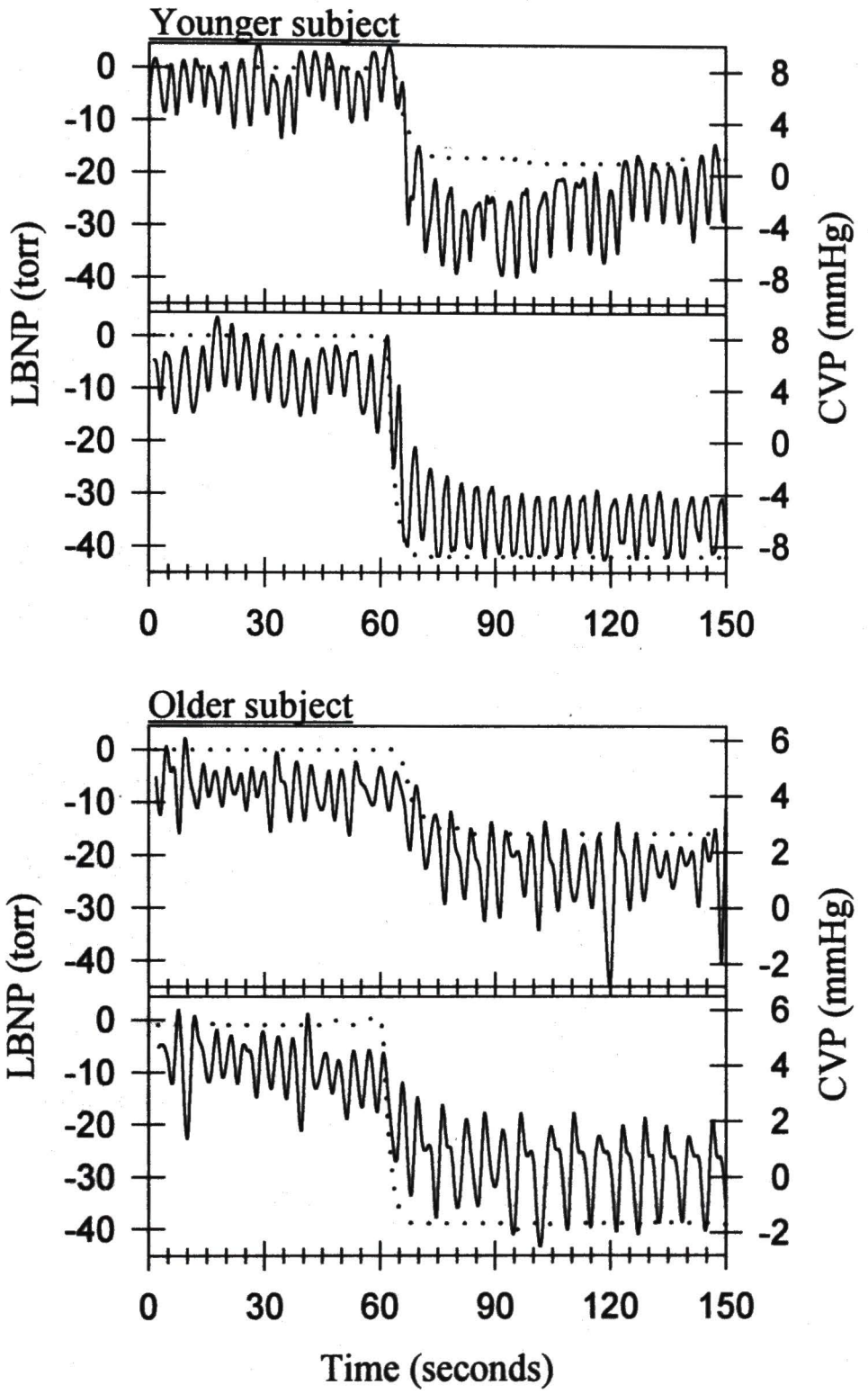

Younger subject
Older subject
LBNP (torr)
CVP (mmHg)
0
-10
-20
-30
-40
8
4
0
-4
-8
6
4
2
0
-2
0
30
60
90
120
150
Time (seconds)

Figure 2

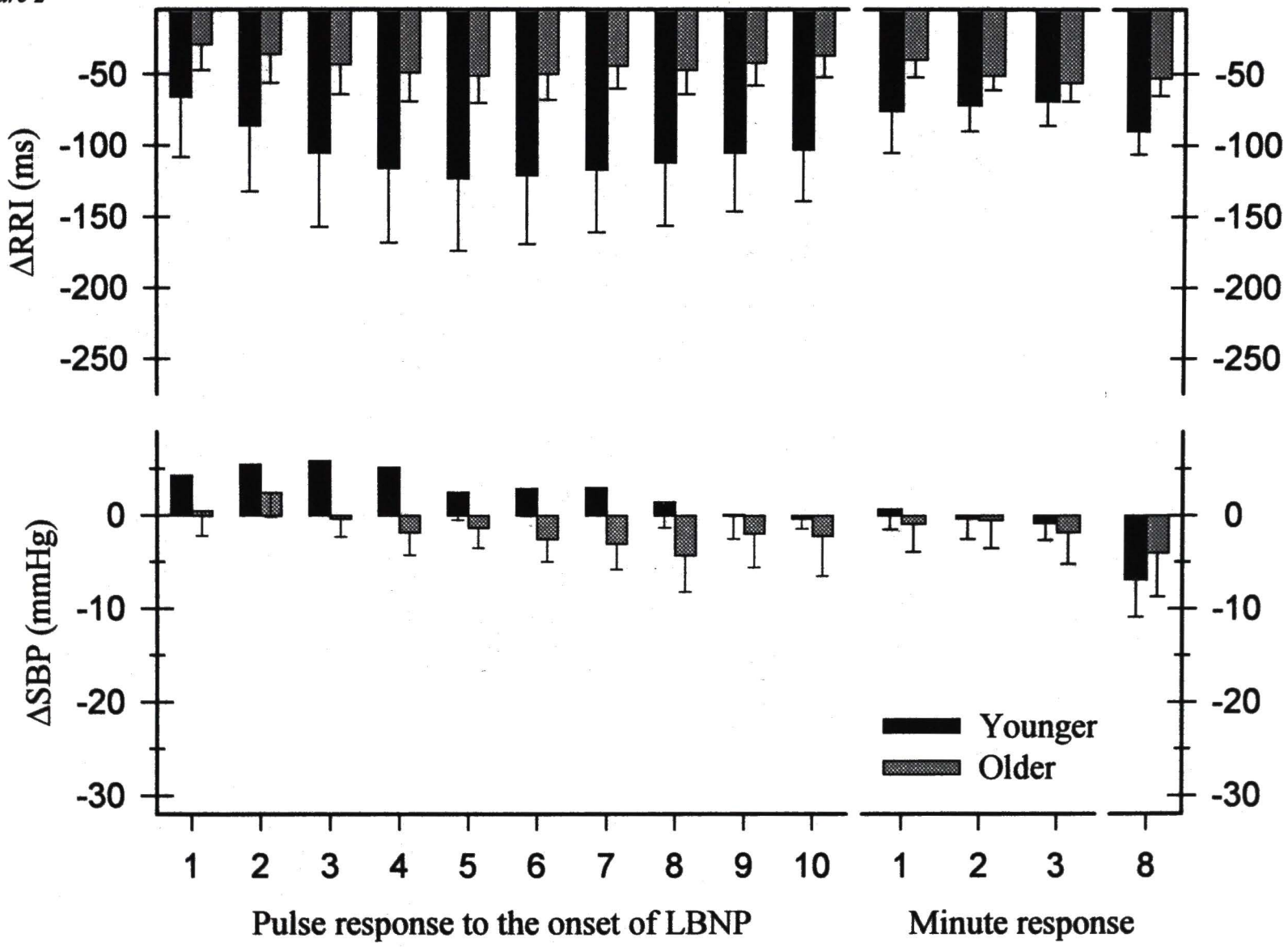
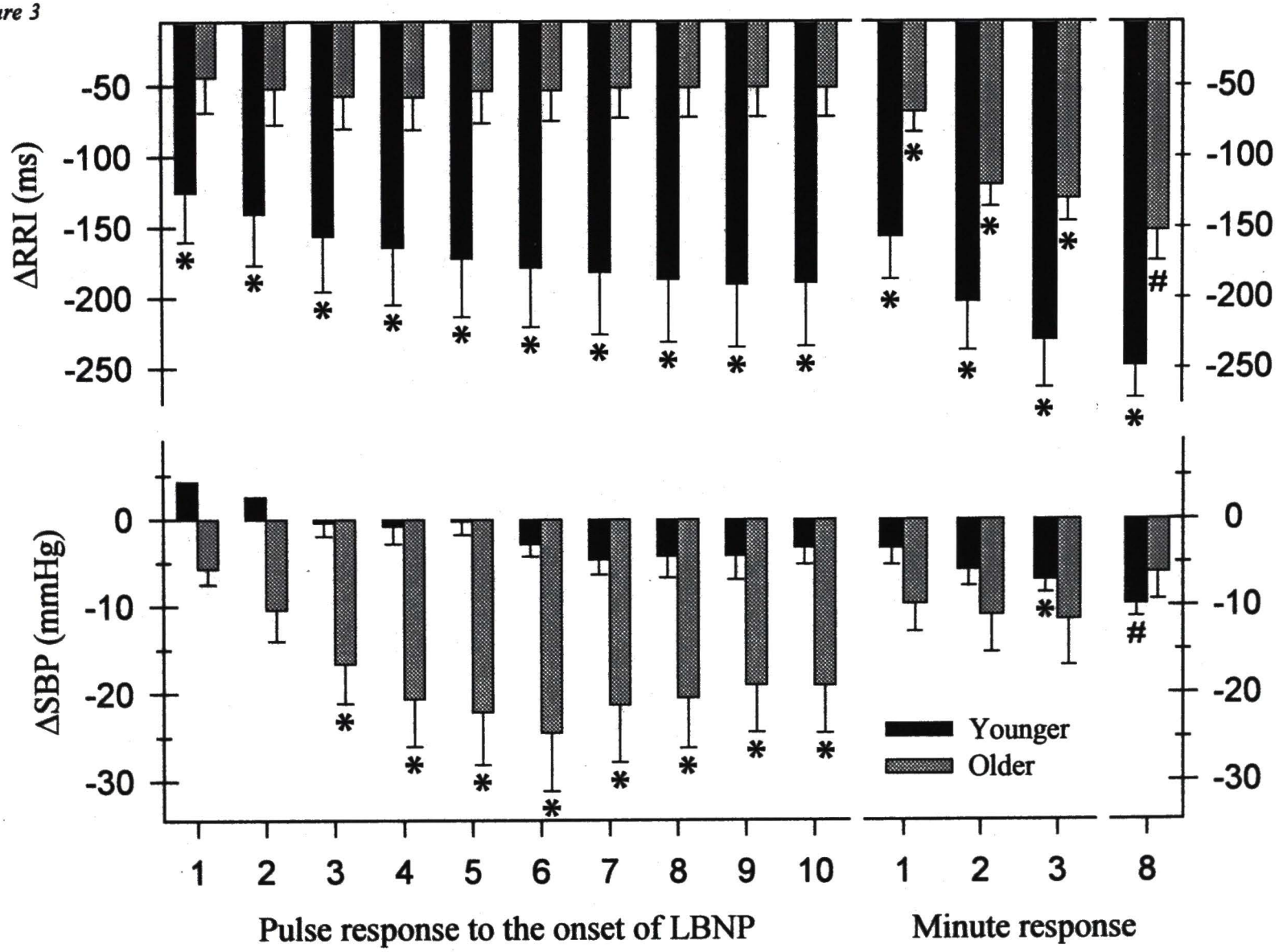

ΔRRI (ms)
-50
-100
-150
-200
-250
ΔSBP (mmHg)
0
-10
-20
-30
Younger
Older
1
2
3
4
5
6
7
8
9
10
1
2
3
8
Pulse response to the onset of LBNP
Minute response

Figure 3


ΔRRI (ms)
-50
-100
-150
-200
-250
ΔSBP (mmHg)
0
-10
-20
-30
Younger
Older
1
2
3
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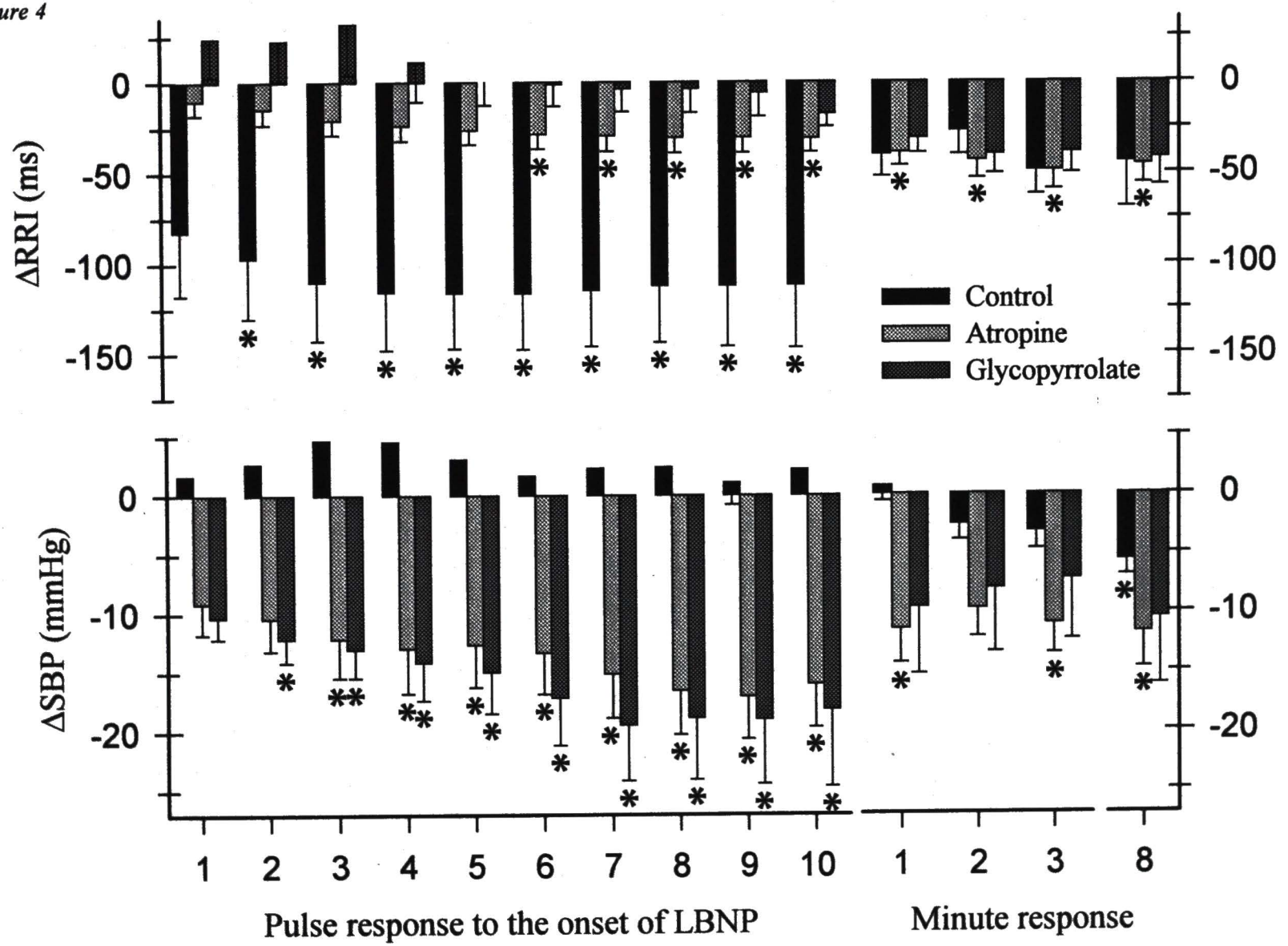
8
9
10
Pulse response to the onset of LBNP
1
2
3
8
Minute response

Figure 4


ΔRRI (ms)
0
-50
-100
-150
Control
Atropine
Glycopyrrolate
ΔSBP (mmHg)
0
-10
-20
1
2
3
4
5
6
7
8
9
10
Pulse response to the onset of LBNP
1
2
3
8
Minute response

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IMPORTANCE OF VAGAL-CARDIAC INFLUENCE IN ARTERIAL BLOOD PRESSURE REGULATION: A COMPARISON OF THE MUSCARINIC CHOLINERGIC ANTAGONISTS ATROPINE AND GLYCOPYRROLATE

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ABSTRACT

This study was designed to test the hypothesis that vagal cardiac modulation plays an important role in arterial blood pressure (ABP) regulation. Changes in heart rate (HR) and ABP (measured by intra-radial arterial catheter or radial tonometry) were assessed in ten healthy young (22±1.75 yrs) volunteers at rest and during graded lower body negative pressure (LBNP) before and after atropine or glycopyrrolate administration. Transient hypertension was induced both by phenylephrine (PE) (1μg/kg body weight) and release of the Valsalva maneuver, while hypotensive challenge was induced by bilateral thigh cuff deflation following a 3-min supersystolic occlusion. Power spectral density of systolic (SBP) and diastolic (DBP) ABP variability was examined at rest and during LBNP –40 and –50 torr. Atropine and glycopyrrolate elicited a baseline tachycardia without significant alteration of baseline ABP. Increases in SBP following injection of PE (before vs. after vagal blockade: +14.3±1.6 vs. 27.2±2.8 mmHg) and Valsalva release (+26.1±1 vs. +51.4±12 mmHg) were significantly augmented after atropine or glycopyrrolate administration, associated with a diminished bradycardiac response. In addition, the decrease in SBP following cuff deflation was significantly greater (-9.2±1.2 vs. -14.5±0.9 mmHg) after vagal blockade, with near abolition of tachycardia. LBNP decreased SBP (P=0.001) and increased HR (P=0.001) before and after vagal blockade. However, after vagal blockade the reduction in SBP was greater (P=0.041), associated with significantly less tachycardia. Vagal blockade tended to reduce the low frequency (LF) power of SBP (P=0.024) and DBP (P=0.035) variability at rest, and the reduction appeared greater with glycopyrrolate than with atropine. Application of LBNP increased LF power of SBP (P=0.01) and DBP (P=0.04) variability, which was significantly augmented following atropine and glycopyrrolate administration. We found that vagal blockade with atropine or glycopyrrolate compromised hemodynamic homeostasis, reflecting the importance of cardiac parasympathetic influence in ABP regulation.

Key words: *blood pressure variability, lower body negative pressure, pressor response, Valsalva maneuver, phenylephrine, power spectral analysis.*

INTRODUCTION

Both sympathetic and parasympathetic branches of the autonomic nervous system contribute to heart rate (HR) regulation (18, 24, 29-31). It has been demonstrated that the bradycardiac response (prolonged pulse interval) at rest is predominately mediated by vagal activation, and that the tachycardiac response is due to both sympathetic activation and vagal inhibition (18). The immediate response of this vagal withdrawal is especially important for initiation of "reflex tachycardia". We have recently identified a greater decrease in arterial blood pressure (ABP) associated with a diminished tachycardiac response at the onset of lower body negative pressure (LBNP) in the elderly compared to young adults (9). This attenuated tachycardiac reflex at the onset of orthostatic challenge results primarily from an age-related change in vagal control of HR, as indicated by the diminished high frequency (HF) power spectrum of HR variability (34). When the muscarinic cholinergic (MC) receptor antagonists atropine or glycopyrrolate were administered to young subjects to simulate vagal dysfunction, their tachycardiac response to the onset of LBNP was nearly abolished, associated with a significant hypotension, a response similar to that seen in elderly individuals (35). However, the impact of MC receptor blockade on ABP regulation during sustained orthostatic challenge has yet to be established. In addition, the question remains as to whether pharmacologically simulated vagal dysfunction compromises ABP regulation during *hypertensive* challenge.

The purpose of this study was to investigate hemodynamic regulation during sustained LBNP by considering the role of baroreflex responsiveness in maintaining ABP stability during hypotensive and hypertensive stimuli before and after MC receptor blockade. We recruited only

young, healthy subjects (20-30 years of age) and performed pharmacological *simulation* of vagal dysfunction with the MC receptor antagonists atropine and glycopyrrolate. Atropine is known to penetrate the blood brain barrier, thus blocking both central and peripheral MC receptors (1), while glycopyrrolate has little influence on the central nervous system (4). We compared the cardiovascular responses and baroreflex function using equipotent (3, 5) doses of the two drugs. We hypothesized that use of either muscarinic antagonist would simulate vagal dysfunction and compromise ABP regulation, but that the degree of change in ABP regulation with atropine would differ from glycopyrrolate.

METHODS

Subjects. Ten disease and drug-free men (n=7) and women (n=3) volunteers (22±1.75 yrs) were recruited as subjects. On a preliminary screening day, all subjects completed a medical history questionnaire, provided a resting twelve-lead electrocardiogram (ECG), and performed a graded exercise test to volitional fatigue for maximal oxygen consumption (VO_{2max}) assessment. Women subjects were excluded from the study if they were pregnant. Eligible subjects received both oral and written explanation of the experimental protocol, and signed a written consent form that was approved by the Institutional Review Board for the Protection of Human Subjects at the University of North Texas Health Science Center at Fort Worth.

Techniques of Measurement. During each experiment, beat-to-beat heart rate (HR) and arterial blood pressure (ABP) signals were digitized (Vetter digital 3000) and collected using an online computer (Gateway2000, 486DX). The lead II ECG signal was used to monitor HR on an electrocardiograph (Hewlett Packard 78342A). On experimental day one, an ABP signal was established using intra-radial arterial catheterization. Catheter insertion was performed by a

licensed cardiologist, using local anesthesia to minimize subject discomfort. During experimental day two, indirect radial tonometry (Colin 7000) was utilized. Previous data (13) indicated a close correlation between tonographic and intra-ABP measurements. Our laboratory observations indicated that carefully monitored indirect measurements of radial arterial pressure by tonography were highly correlated with intra-radial arterial catheter measurements (figure 1). A venous catheter was inserted into the subject's median antecubital vein for intravenous drug injections. Stroke volume (SV) and cardiac output (CO) values were collected by impedance plethysmography (Minnesota Impedance Cardiograph) using Z null (ohm) values. Peripheral vascular resistance (PVR) was then calculated from the equation PVR = MAP/CO.

Protocol. Before each experiment, subjects were familiarized with the procedures and measurements to be used during the test. All experiments were carried out with the subject supine in the lower body negative pressure (LBNP) chamber, with an ambient temperature of 24-26°C. The subjects were encouraged to lay perfectly still and not initiate any contraction in the leg muscles (32). After 30 minutes of rest, baseline values for HR, ABP, SV, and CO were recorded. Baseline included 10 minutes with metronomic breathing at 15 cycles per minute, followed by 10 minutes without breath control. At the end of each ten-minute interval, bilateral thigh cuffs were inflated to ≥200 mmHg for three minutes. Phenylephrine (PE) was then administered at 1.0μg/kg body weight to create transient hypertension. In addition, five subjects were asked to perform a Valsalva maneuver. The HR and ABP responses following cuff deflation, Valsalva, and PE were used for assessment of baroreflex sensitivity and it's effect on ABP regulation.

When ABP returned to baseline, a graded LBNP test was conducted. The protocol involved 10 minutes at -40 torr, -50 torr and -60 torr, or until maximal tolerance was reached. At the end

of LBNP –40 torr, arterial blood samples were taken for (EPI) and (NE) levels, and bilateral thigh cuffs were again inflated for three minutes. Following graded LBNP, the subject was released from the box and allowed to rest for ≥45 minutes to allow recovery. After the subject returned to the LBNP box, either atropine or glycopyrrolate was injected intravenously (5.0μg/kg or 2.0μg/kg, respectively) to achieve full vagal blockade. Injections were administered over several minutes until the subject's heart rate achieved a plateau, such that two consecutive doses did not yield any additional increase in HR, or until the maximum allowable dosage (40 μg/kg or 16μg/kg, respectively). Upon completion of drug injection, the above protocol was repeated in its entirety. After the experiment was concluded, the subject was observed for ≥30 minutes to ensure full recovery.

On the second experimental day, the subject received either atropine or glycopyrrolate, whichever drug was not used on day one, and the day one protocol was repeated. Venous blood was collected during baseline and LBNP before and after drug administration for measurement of plasma catecholamines. Each 5ml sample was centrifuged for 15 min and frozen at -90°C, and the supernatant was analyzed using high-pressure liquid chromatography (HPLC) within two weeks.

Data Management. Power spectral analysis was used to estimate autonomic balance (see appendix I). The principles of the software for data acquisition and analysis have been described elsewhere (11, 19, 21). From each ten minute data set, the best six minutes of beat-to-beat pulse interval (RRI), HR, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were converted with fast Fourier transformation (17) using commercially available software (DADiSP/AdvDSP, Cambridge, MA). Typical power spectral density curves for RRI and SBP variability are shown in figure 2. Harmonic power in both high (HF) and low frequency (LF) was

considered in determination of autonomic influence on HR and ABP. The low frequency band was defined from 0.04 to 0.12 Hz and the high frequency band from 0.20 to 0.28 Hz. HR variability was reported as absolute, normalized (NU, i.e. LFNU = LF/(LF+HF) * 100), and ratio (LF/HF) to assess the fractional distribution of power across the frequency axis. LF power of ABP variability was analyzed as an index of sympathetically mediated vasomotor modulation (22). The magnitude of the transfer function of simultaneous SBP and RRI variability was assessed as an index of baroreflex gain (21).

Data are reported as group means ± the standard error of the mean (SE). Analysis of variance (ANOVA), analysis of covariance (ANCOVA), t-test, or simple/multiple linear regression analysis was used to test for significance. In testing for significant differences for multiple comparison of the means (post-hoc analysis), Duncan's method was used. Statistical analysis was conducted with statistic analysis system (SAS) software. Significance was set at $P<0.05$ for all statistical tests.

RESULTS

Baseline values. Baseline heart rate (HR) increased and pulse interval (RRI) decreased significantly after both atropine and glycopyrrolate (table 1), with no difference between the drug treatments. Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and pulse pressure (PP) were similar before and after drug administration. There was no significant difference in cardiac output (CO) after either atropine (n=5) or glycopyrrolate (n=3) administration. Since there was no observed drug difference and sample size was small, CO and peripheral vascular resistance (PVR) data were merged and classified as "before" and "after cholinergic blockade" groups. Drug administration did not significantly change CO (7.0±0.3 vs. 6.1±0.2 l/min), although stroke volume (SV) decreased significantly from a baseline of 113±8.5

to 58.3±2.8 ml/beat following drug administration. PVR tended to increase after blockade (12.8±0.7 to 14.9±0.7 PRU, P=0.06). During baseline and lower body negative pressure (LBNP) –40 torr, epinephrine (EPI) and norepinephrine (NE) values were not significantly different after cholinergic blockade or between drugs (table 2).

Low frequency (LF) RRI variability was significantly less than control after both drugs (table 3). The response to glycopyrrolate was significantly less than atropine (P=0.04). High frequency (HF) power was almost completely abolished after blockade. Surprisingly, metronomic breathing failed to show any significant differences in baseline HR variability (LF 288±84 ms^2/Hz, HF 29.4±15.9 ms^2/Hz with breath control at 15 cycles/min). LF power of SBP and DBP was reduced after muscarinic cholinergic (MC) blockade, but was significantly less than control only with glycopyrrolate (Duncan post-hoc analysis, see table 4). Following MC blockade, LF power was consistently smaller with glycopyrrolate than with atropine treatment for both systolic and diastolic LF variability (P=0.01). HF power of DBP variability was greater with atropine (P=0.09), but significantly greater than control only with glycopyrrolate. HF SBP failed to reach significance among the three conditions.

Baroreflex gain. Following cuff release, the ΔSBP was greater than control (-9.2±1.2 mmHg, n=9) after atropine (-14.5±0.8 mmHg, n=6) and glycopyrrolate (-14.3±2.2 mmHg, n=6). However, the tachycardiac response to the ΔSBP following cuff release was significantly less than control (10.6±1.9 ms/mmHg) after atropine (0.9±0.1 ms/mmHg) and glycopyrrolate (0.6±0.1 ms/mmHg). In addition, the baroreflex slope of changes in RRI to SBP during phenylephrine (PE) injection was significantly less than control (15.0±3.5 ms/mmHg, n=9) after atropine and glycopyrrolate (1.3±0.6 and 1.3±0.7 ms/mmHg, respectively, n=6 for both groups). However, the hypertensive response, in terms of the rate of increase in SBP per unit time

(ΔSBP/*t*), to the same dose of PE was significantly augmented after vagal blockade (control: 0.8±0.2 mmHg/s, atropine: 2.0±0.2 mmHg/s, and glycopyrrolate: 2.2±0.5 mmHg/s). Figure 3 illustrates individual changes in heart rate (HR) and mean arterial pressure (MAP) following release of the Valsalva maneuver before and after atropine administration. The reflex tachycardiac response (change in pulse interval per unit time, ΔRRI/*t*) was significantly diminished following vagal blockade (40.6±7.0 ms/s vs. 6.8±1.3 ms/s, n=5), associated with an augmented ΔSBP (before blockade: +26±1.9 mmHg, after blockade: +51.4±12.0 mmHg).

LBNP values. SBP decreased and DBP was maintained during LBNP (table 1), and the decrease in SBP tended to be greater (P<0.05) after vagal blockade. However, this change in SBP was not statistically different between atropine and glycopyrrolate. Compared to control, less reflex tachycardia was observed with both drug groups during LBNP. Figure 4 illustrates the changes in CO in all groups during minutes 2, 5, and 8 of LBNP –40 and –50 torr, associated with a decrease in MAP (left panel). Though the changes in CO and MAP were consistently greater after vagal blockade, the rate of decrease in MAP per unit decrease in CO was similar to baseline values (slope = 0.43 for both groups). In addition, a greater decrease in CO with a reduced tachycardia was observed after vagal blockade (right panel). LBNP –40 torr significantly increased NE levels in all groups, with the atropine group showing higher post-drug Δ[NE] levels than glycopyrrolate (108±25 vs. 49±26%, respectively, P=0.18). EPI levels did not increase significantly during LBNP in any group (table 2).

Values for the absolute and normalized variance of RRI before and during graded LBNP are given in table 3. For both HF and LF, drug administration significantly reduced RRI variability at baseline and LBNP -40 and -50 torr. Normalized high frequency (HFNU) showed a progressive decline while normalized low frequency (LFNU) showed a progressive increase during LBNP to

–50 torr, with similar trends among all groups (P=0.06). Drug administration did not significantly increase the absolute LF variability of systolic (P=0.11) or diastolic (P=0.14) pressure during LBNP (table 4). However, LF SBP variability was greater with atropine than glycopyrrolate at rest (P=0.01) and during –40 torr (P=0.11). Drug administration significantly reduced the magnitude of the transfer function of SBP to RRI at all LBNP levels, with no difference between atropine and glycopyrrolate.

DISCUSSION

This study demonstrated that the hypertensive response following phenylephrine (PE) injection and Valsalva strain release was significantly augmented after muscarinic cholinergic (MC) blockade with either atropine or glycopyrrolate, accompanied by a substantially diminished reflex bradycardia. In addition, the hypotensive response to post-ischemic cuff release was also augmented after vagal blockade, associated with a reduced reflex tachycardia. During sustained lower body negative pressure (LBNP) –40 and –50 torr, the tachycardiac response was less significant, and low frequency (LF) systolic (SBP) and diastolic (DBP) blood pressure variability tended to be greater following atropine or glycopyrrolate administration. These data indicate that MC receptor blockade compromised arterial blood pressure (ABP) regulation during both hypotensive and hypertensive stimuli.

Response to hypertensive and hypotensive challenge. The heart rate (HR) response to a transient fall in ABP involves both parasympathetic (immediate) and sympathetic (delayed) modulation (6). In this study, the importance of vagal function in immediate ABP regulation was demonstrated by evaluation of baroreflex gain. After cholinergic blockade, baroreflex sensitivity fell dramatically. Without the buffering effect of reflex HR changes, changes in systolic blood

pressure (ΔSBP) in response to both pressor and depressor stimuli were much greater. This lack of appropriate HR compensation is similar to that seen in the elderly (9, 25), supporting the hypothesis that MC receptor blockade simulates the age-related diminution of vagal function in young subjects.

Effects of MC receptor blockade on sustained LBNP response. While the parasympathetic reflex dominates the immediate HR response (25, 27), adequate sympathetic activation is essential to the maintenance of ABP during prolonged orthostatic stress. A reduction in reflex tachycardia was observed during prolonged orthostatic stress after drug administration. Clearly, augmentation of vasomotor tone was necessary to prevent orthostatic hypotension in these individuals. Figure 5 illustrates the percent changes in mean arterial pressure (MAP) and cardiac output (CO) during LBNP. While the blockade group experienced a greater change in CO than control, MAP was remarkably well maintained due to an increase in peripheral vascular resistance (PVR), indicated by similar slope values between the groups.

Spectral analysis of systolic and diastolic blood pressure variability was used to further assess vascular sympathetic activity during orthostatic stress. Recent studies have confirmed that the low frequency (LF) power of ABP variability is predominately caused by fluctuations of vasomotor tone and systemic vascular resistance (22), and thus can be used to provide an index of sympathetic activity. During graded LBNP, absolute LF power of SBP appeared greater after drug administration (P=0.08). Furthermore, the change in LF SBP from baseline to –40 torr was dramatically greater after parasympathetic blockade. These data agree with evidence that an increase in vasomotor tone occurred in response to orthostasis (23), and suggest that this compensatory response was necessarily augmented after parasympathetic blockade. After MC blockade, PVR was greater at rest (P=0.06) and during LBNP (P<0.05), further indicating an

increase in the vasoconstrictor response. These compensations were necessary to maintain adequate ABP in the face of a reduced tachycardiac response.

Changes in pulse interval (RRI) variability during sustained LBNP were less conclusive. Absolute high frequency (HF) power of RRI was significantly less after autonomic blockade, verifying the abolition of vagal control. LF power provides an index of both sympathetic and parasympathetic control (2), and was likewise significantly reduced after drug administration. As expected, graded LBNP significantly increased LF and LF/HF power, confirming an increase in sympathetic outflow to the heart with LBNP. Unfortunately, the absolute sympathetic activity (LF/HF) could not be verified after MC blockade. Thus, it appears that during sustained orthostatic stress the most significant effects of parasympathetic blockade are seen in the augmentation of vasomotor tone.

Central versus peripheral MC receptor blockade. Contrary to our hypothesis, no statistically significant differences were seen between atropine and glycopyrrolate in the maintenance of ABP at rest or during graded LBNP. However, several interesting trends emerged concerning the central involvement in baroreflex signaling. Absolute LF power of SBP was greater with atropine than glycopyrrolate at rest (1.67±0.35 vs. 0.485±0.17 ms^2/mmHg, P=0.01), but was not significantly greater during LBNP –40 torr (6.48±2.09 vs. 2.40±0.68 ms^2/mmHg, P=0.11). In addition, the percent change in norepinephrine concentration (%Δ[NE]) in response to LBNP –40 torr with atropine was twice as great as glycopyrrolate (108.2±25.0 vs. 48.5±26.0%, P=0.18). These data seem to indicate an increased sympathetic activation after atropine administration, suggesting acetylcholine (ACh) and the MC receptors play a role in central autonomic regulation.

Several recent studies addressing the variety of neurotransmitters and receptors involved in the integration of afferent signals emphasize the importance of the nucleus tractus solitarius

(NTS) in regulation of the medullary cardiovascular center (14). Quantitative receptor autoradiography has confirmed high MC receptor density in the NTS of cats (16) and humans (7, 10). After central MC receptor blockade in rats, significant increases in ABP have been observed, indicating cholinergic input to the NTS modulates the responsiveness of reflex interneurons (15). Criscione *et al* (8) suggests the central MC receptors are tonically activated, lowering ABP through a decrease in peripheral sympathetic activity. Their data indicated that the integrity of the baroreceptor reflex is not interrupted by vagal blockade at the NTS with atropine, again suggesting the cholinergic system modulates the baroreceptor reflex. Our data support the role of central MC receptors in altering the fine control of ABP regulation.

Baroreflex gain in time and frequency domains. Baroreflex responsiveness was quantified with several equally effective indices. Valsalva and thigh occlusion techniques have been widely used to measure baroreflex mechanisms in the time domain, using the slope of the linear relationship between SBP and RRI as a measure of baroreflex sensitivity (26, 28). Our data agree with other studies (33) in demonstrating a significant decrease in baroreceptor sensitivity after parasympathetic blockade. The magnitude of the transfer function of SBP to RRI provides an additional means of assessing the overall baroreflex gain in the frequency domain, based on a closed loop model (20). The magnitude of the HF transfer function was decreased with LBNP during control (no drug) conditions. After atropine or glycopyrrolate, transfer function values were significantly less, with coherence values well below acceptable levels. These data are in agreement with the open loop reflex gain values assessed by Valsalva and cuff release before and after MC blockade. Thus, our findings agree with others (20) in support of spectral analysis as an accurate, noninvasive indicator of baroreflex sensitivity.

Summary. Our findings emphasize the importance of HR reflex sensitivity in the regulation of ABP, and support the theory that this reflex gain is primarily determined by vagal function. Both MC antagonists significantly compromised regulation of ABP during hypotensive and hypertensive stimuli. During sustained orthostatic stress, low frequency SBP and DBP variability was augmented after vagal blockade, yet augmentation of vasomotor modulation effectively compensated for the diminished vagal function. These responses are similar to those experienced by the elderly population, supporting the use of parasympathetic blockade to mimic age-related vagal dysfunction. No significant changes were noted between central and peripheral blockade. However, atropine tended to increase several indices of sympathetic nerve activity (SNA). Increased SNA is often observed in the elderly, suggesting a decline in functional central integration may be partially responsible for the attenuation of baroreflex function with age.

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APPENDIX I

Spectral analysis methods are quickly evolving as a useful tool in physiological research. While multivariate models may pose problems concerning accurate interpretation and physiological significance, the interactions between changes in heart rate (HR) and arterial blood pressure (ABP) are increasingly employed in assessment of cardiovascular regulation (21).

Fast Fourier transformation of a given time series provides an autospectrum representing the power distribution of the signal (see figure 2). For data analysis, the fractional distribution of power across the frequency axis can be divided into separate components. For arterial blood pressure, low frequency (LF) power provides an index of sympathetic modulation, and is thus used as an indicator of vasomotor activity (22). The physiological significance of high frequency (HF) power of ABP is not well understood. For heart rate, LF power values are influenced by both sympathetic and parasympathetic activity. However, HF power gives an estimation of purely parasympathetic (vagal) activity (11). In addition to these "absolute" values, harmonic power can be converted to normalized units (NU). The ratio of low and high frequencies (LF/HF) is commonly calculated for HR power as an index of exclusively sympathetic activity (12). However, this calculation is not appropriate when the denominator becomes extremely small, such as during parasympathetic blockade.

In this study, a multivariate model was established to consider both HR and ABP variability, and how these two integrated cardiovascular components interact under various conditions. The transfer function (gain) reflects the relationship between HR and ABP variability by comparing the relative amplitude of the two signals over a specific frequency range. The reliability of this estimation is evaluated using the magnitude-squared coherence function. A coherence value near 1 (unity) suggests a linear relationship between two variables, while a value near zero implies no

relationship between two variables. With adequate coherence, the magnitude of the transfer function of ABP to HR provide an index of baroreflex sensitivity, based on the so-called "closed loop" model (21).

FIGURE LEGEND

Figure 1: Individual subject beat-to-beat systolic and diastolic blood pressure measured by radial tonography and an intra-arterial catheter during one minute baseline and lower body negative pressure (LBNP) –50 torr conditions.

Figure 2: Individual subject data to provide representative curves for power of pulse interval (RRI) and systolic blood pressure (SBP) variability before (solid line) and after (dotted line) glycopyrrolate.

Figure 3: Representative subject data showing mean arterial pressure (MAP), pulse interval (RRI), and thoracic impedance (Z null) during a Valsalva maneuver, performed during control (left panels) and vagal blockade (right panels) conditions.

Figure 4: Changes in CO in a group of young adults (n=7) during minutes 2, 5, and 8 of LBNP –40 and –50 torr, associated with a decrease in MAP (left panel). Though the changes in CO and MAP were consistently greater after vagal blockade, the rate of decrease in MAP per unit decrease in CO (slope) was similar to baseline values (0.43mmHg/l/min). DL1 indicates a slope corresponding to "perfect vasoconstriction", while DL2 represents "no vasoconstriction". A greater decrease in CO with a reduced tachycardia was observed after vagal blockade (right panel). Solid and hollow circles indicate before and after vagal blockade, respectively.

Table 1: Cardiovascular variables during supine rest and during graded LBNP before and after drug administration.

Variables	RRI (ms)	HR (bpm)	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)	PP (mmHg)
Control						
Baseline (n=10)	1048±58.8	59±3.1	123±2.5	66±1.8	85±2.2	57±1.3
Δ -40 torr (n=10)	-58±20.2	3.8±1.4	-3.1±1.1	1.8±1.0	0.2±0.9	-4.9±1.4
Δ -50 torr (n=8)	-155±32.3†	12±3.1†	-8.7±1.6†	2.0±1.2	-1.4±1.1	-11±1.8†
Δ -60 torr (n=6)	-287±66.1†	21±4.2†	-11.4±3.6†	3.7±2.1	-1.3±2.7	-15±2.3†
Atropine						
Baseline (n=6)	591±31.2‡	103±5.5‡	130±3.2	72±2.9	91±2.7	59±1.7
Δ -40 torr (n=6)	-41±8.9†	7.5±1.4†	-12±1.8	-0.3±1.8	-3.3±1.3	-11±0.9†‡
Δ -50 torr (n=4)	-35±7.1	7.4±2.3	-17±3.8†	-1.45±2.7	-5.7±2.4	-16±1.1†
Glycopyrrolate						
Baseline (n=6)	548±18.1‡	110±3.6‡	129±5.4	71±4.3	90±4.7	58±1.4
Δ -40 torr (n=6)	-27±16.8	6.7±3.3	-11±4.6	1.9±3.8	-0.6±2.7	-13±1.1†‡
Δ -50 torr (n=4)	-15±23.1‡	4.9±4.9	-15±4.8	1.3±4.6	-3.5±5.4	-16±1.9†

Values are mean ± SE, averaged over six minutes of continuous data. After atropine or glycopyrrolate, only one subject in each group reached LBNP –60 torr (data not included). † indicates $P<0.05$ vs. baseline within the same group, ‡ indicates $P<0.05$ vs. control group, same LBNP level.

Table 2: Plasma [EPI] and [NE] during baseline and –40 Torr LBNP.

Variable	Control	Atropine	Glycopyrrolate
[EPI] Baseline (pmol/ml)	0.43±0.18	0.22±0.07	0.38±0.12
–40 torr (pmol/ml)	0.39±0.08	0.18±0.05	0.30±0.00
[NE] Baseline (pmol/ml)	1.42±0.21	1.18±0.33	1.21±0.11
–40 torr (pmol/ml)	2.05±0.27†	2.44±0.78†	1.63±0.28†

Values are mean ± SE (n=10 control, n=6 atropine, and n=4 glycopyrrolate).

† indicates $P<0.05$ vs. baseline.

Table 3: RRI variability at rest and during graded LBNP.

	LF	LFNU	HF	HFNU	LF/HF
Control					
Baseline (n=10)	386±92.1	0.937±0.01	19.9±6.5	0.063±0.02	41.4±14.4
-40 torr (n=10)	228±42.9	0.951±0.02	9.81±4.5	0.049±0.02	46.8±12.2
-50 torr (n=8)	393±61.5	0.972±0.02	6.79±3.9	0.028±0.02	214±97.2
-60 torr (n=6)	648±150	0.993±0.00	3.26±0.9	0.007±0.00	469±292
Atropine					
Baseline (n=6)	6.06±1.6‡	0.948±0.03	0.19±0.1‡	0.052±0.03	65.6±28.0
-40 torr (n=6)	11.8±3.4‡	0.984±0.01	0.18±0.1‡	0.016±0.01	129±45.1
-50 torr (n=4)	60.5±41.9‡	0.981±0.01	0.46±0.4‡	0.019±0.01	175±86.0
Glycopyrrolate					
Baseline (n=6)	1.94±0.6‡¥	0.955±0.01	0.09±0.1‡	0.045±0.01	25.9±5.1
-40 torr (n=6)	5.79±1.9‡	0.965±0.01	0.12±0.0‡	0.035±0.01	55.2±19.5
-50 torr (n=4)	15.4±8.5‡	0.976±0.01	0.15±0.0‡	0.024±0.01	143±88.8

Values are mean ± SE, averaged over six minutes of continuous data. After atropine or glycopyrrolate, only one subject in each group reached LBNP –60 torr (data not included).

LF and HF are (ms^2/mmHg), LFNU and HFNU are (% of total power*100).

‡ indicates $P<0.05$ vs. control group, ¥ indicates $P<0.05$ for atropine vs. glycopyrrolate.

Table 4: ABP variability and transfer function during rest and graded LBNP.

	Control		Atropine		Glycopyrrolate	
	LF	HF	LF	HF	LF	HF
SBP						
Baseline	3.1±0.7	0.7±0.1	1.6±0.3	0.7±0.2	0.5±0.2‡¥	1.1±0.2
-40 Torr	2.9±0.5	0.7±0.2	6.5±2.1	1.2±0.4	2.4±0.7	1.1±0.2
-50 Torr	5.6±1.0	1.0±0.2	14.7±8.9	1.0±0.2	3.8±0.7	1.3±0.2
-60 Torr	11±3.2	0.8±0.2	---	---	---	---
DBP						
Baseline	2.9±0.6	0.2±0.0	2.2±0.4	0.3±0.1	0.7±0.3‡¥	0.7±0.2‡
-40 Torr	3.3±0.5	0.2±0.1	7.7±2.0	0.5±0.1	6.1±2.7	1.0±0.4
-50 Torr	4.9±0.8	0.3±0.1	10.8±4.1	1.0±0.3	6.0±1.1	1.0±0.1
-60 Torr	8.1±2.6	0.3±0.1	---	---	---	---
MAG						
Baseline	7.7±1.2	3.4±0.6	1.4±0.2‡	0.3±0.1‡	1.2±0.2‡	0.2±0.0‡
-40 Torr	6.1±0.8	2.1±.04	1.1±0.3‡	0.2±0.1‡	1.1±0.3‡	0.2±0.3‡
-50 Torr	6.6±0.6	1.5±0.4	1.8±0.7‡	0.3±0.2‡	1.6±0.6‡	0.2±0.1‡
-60 Torr	6.8±0.7	1.4±0.3	---	---	---	---
COH						
Baseline	0.55±0.05	0.50±0.06	0.59±0.04	0.26±0.05	0.40±0.09	0.29±0.03
-40 Torr	0.54±0.04	0.47±0.06	0.68±0.05	0.28±0.05	0.51±0.06	0.24±0.04
-50 Torr	0.63±0.06	0.50±0.06	0.70±0.06	0.30±0.07	0.62±0.07	0.28±0.05
-60 Torr	0.71±0.06	0.30±0.05	---	---	---	---

Values are mean ± SE, averaged over six minutes of continuous data. After atropine or glycopyrrolate, only one subject in each group reached LBNP –60 torr (data not included).

‡ indicates $P<0.05$ vs. control group, ¥ indicates $P<0.05$ for atropine vs. glycopyrrolate.

Figure 1

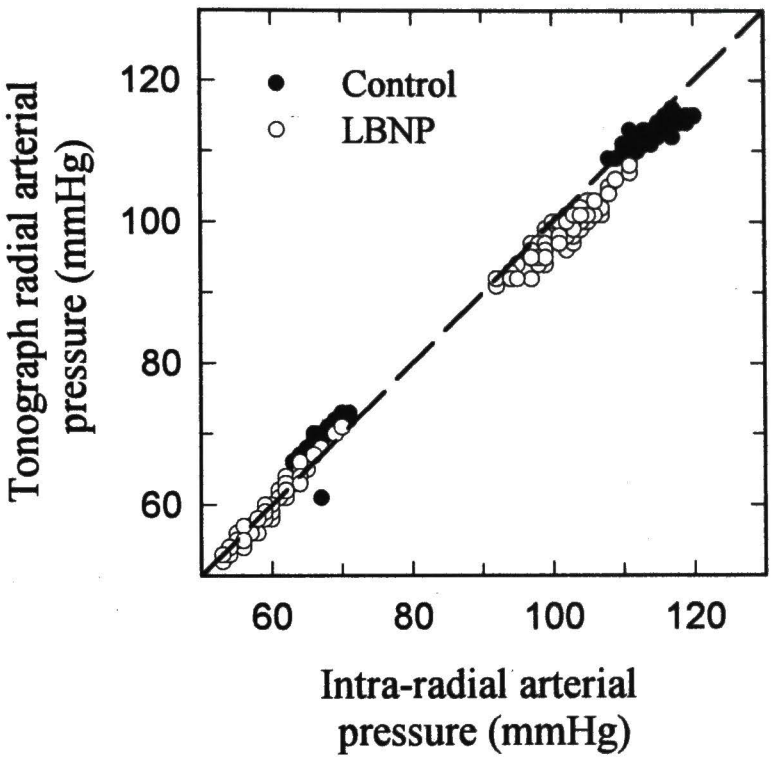

Tonograph radial arterial pressure (mmHg)
120
100
80
60
Control
LBNP
60
80
100
120
Intra-radial arterial pressure (mmHg)

Figure 2

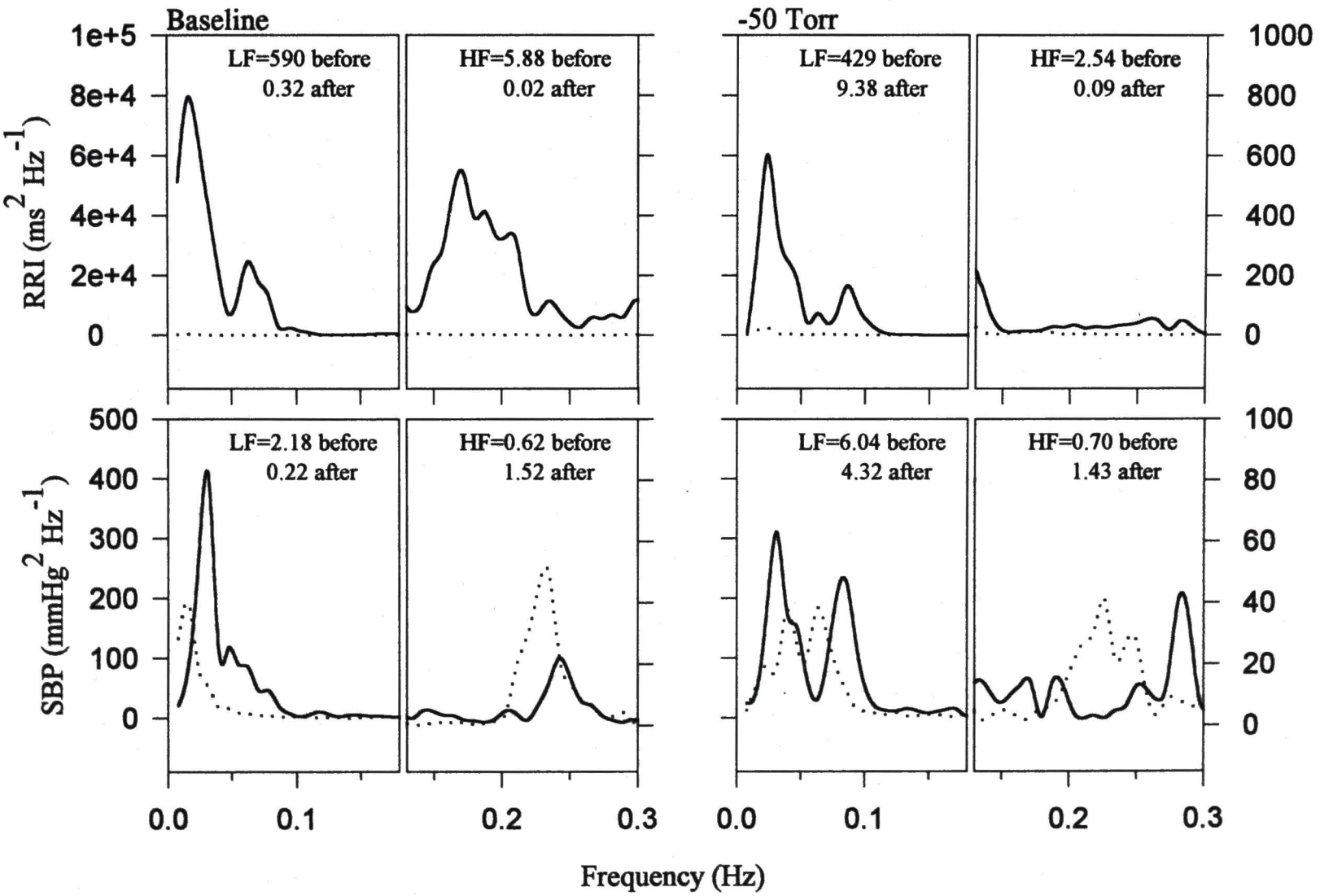

Baseline
-50 Torr
LF=590 before
0.32 after
HF=5.88 before
0.02 after
LF=429 before
9.38 after
HF=2.54 before
0.09 after
LF=2.18 before
0.22 after
HF=0.62 before
1.52 after
LF=6.04 before
4.32 after
HF=0.70 before
1.43 after
RRI (ms^2 Hz^{-1})
SBP (mmHg^2 Hz^{-1})
1e+5
8e+4
6e+4
4e+4
2e+4
0
1000
800
600
400
200
0
500
400
300
200
100
0
100
80
60
40
20
0
0.0
0.1
0.2
0.3
Frequency (Hz)

Figure 3

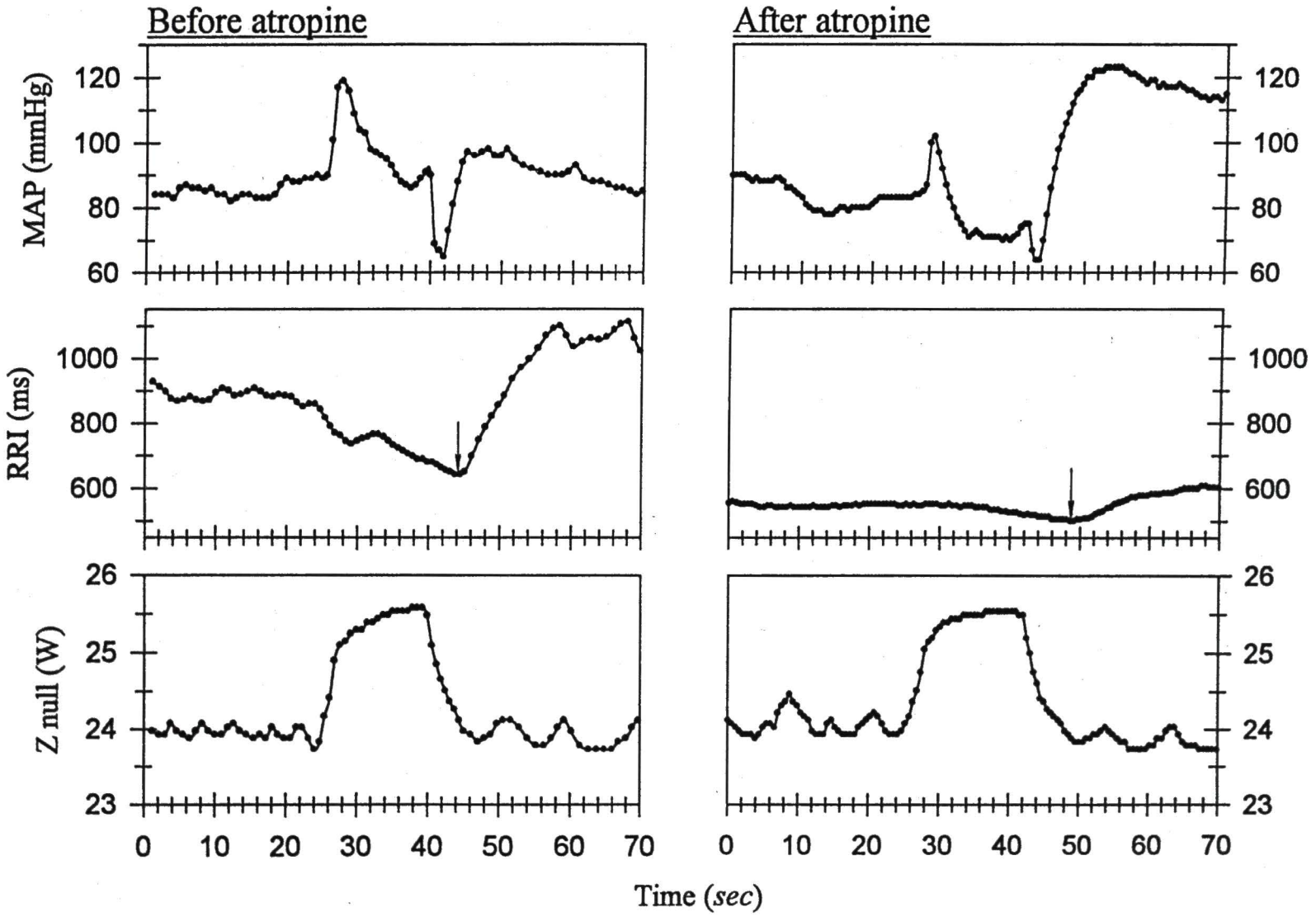
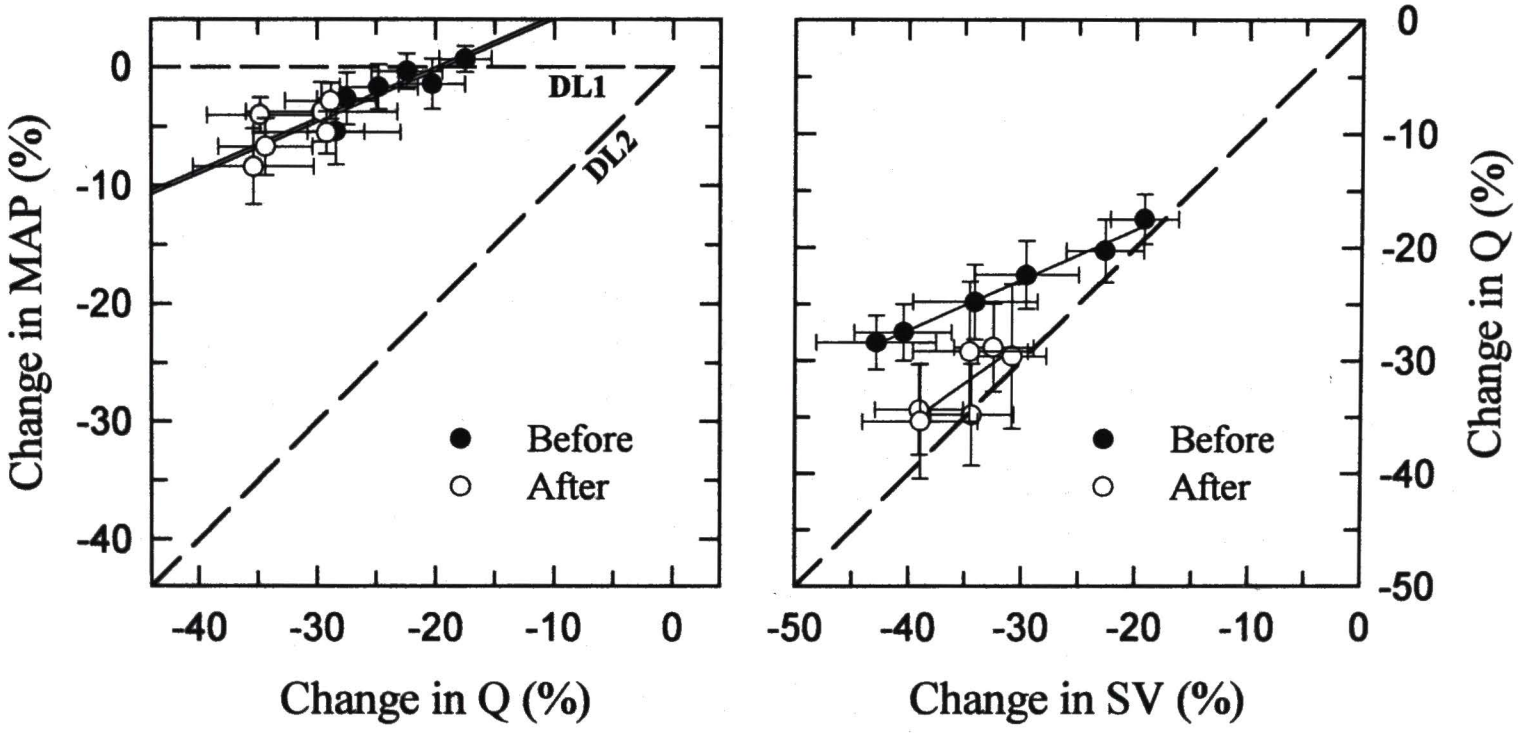

Before atropine
After atropine
MAP (mmHg)
120
100
80
60
RRI (ms)
1000
800
600
Z null (W)
26
25
24
23
0
10
20
30
40
50
60
70
Time (sec)

Figure 4


Change in MAP (%)
0
-10
-20
-30
-40
DL1
DL2
Before
After
-40
-30
-20
-10
0
Change in Q (%)
-50
-40
-30
-20
-10
0
Change in SV (%)
Change in Q (%)
0
-10
-20
-30
-40
-50

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CHAPTER III

CONCLUSION

We compared the reflex heart rate (HR) and arterial blood pressure (ABP) response to lower body negative pressure (LBNP) induced hypovolemia following MC receptor blockade with atropine and glycopyrrolate to assess the central integration of baroreflex signaling. We found that muscarinic cholinergic (MC) blockade compromised ABP regulation during both hypotensive (post ischemic cuff release, onset of LBNP) and hypertensive (phenylephrine injection and Valsalva strain release) stimuli. The differences between central and peripheral blockade were not significant, though several trends in the data suggest central cholinergic activity may be involved in modulation of ABP. These results have led to the following conclusions:

1) MC receptor blockade convincingly demonstrated that HR reflex sensitivity was of paramount importance in the immediate response to changes in ABP, and that it was determined primarily by vagal function. Since blockade appeared to successfully simulate age-related diminution of vagal function, it provides a valuable research tool for aging research.

2) Since MC blockade does mimic age-related changes, it can be implied that the ability of the elderly to tolerate sustained LBNP likely involves augmented vasomotor modulation as a compensatory response to the diminished HR reflex tachycardia. This increase in sympathetic nerve activity (SNA) may be partially attributed to changes in the central integration of baroreflex signaling.

3) Our data suggest that elderly individuals who experience orthostatic intolerance would benefit from improvements in vagal tone. If applicable, the clinician should integrate aerobic exercise into the treatment for these individuals.

CHAPTER IV

PROPOSAL FOR FURTHER RESEARCH

The current study has emphasized the importance of vagal function and central baroreflex integration, and points towards several new ideas for future research. The following areas are suggested for further examination.

1) Analyze the *beat-to-beat* changes in CO and SV during the onset of orthostatic stress to estimate the sympatho-vagal response to a rapid translocation of blood volume.

2) Determine the value of enhanced vagal tone in the elderly. Exercise training studies have the potential to increase vagal activity, which may improve ABP regulation during postural changes for this population.

3) Investigate the vascular receptor sensitivity and intracellular mechanisms that respond to the augmented sympathetic outflow initiated by central cholinergic blockade. This approach might clarify the functional significance of central cholinergic receptors in cardiovascular control.