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L I Sinoway, … , M B Smith, A L Mark

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Research Article

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Hydrogen Ion Concentration Is Not the Sole Determinant of Muscle Metaboreceptor Responses in Humans

Lawrence I. Sinoway,* Robert F. Rea,§ Timothy J. Mosher,‡ Michael B. Smith,‡ and Allyn L. Mark§
*Departments of *Medicine and ‡Radiology, The Milton S. Hershey Medical Center, Pennsylvania State University, Hershey, Pennsylvania 17033; and Cardiovascular and Clinical Research Center, and Department of §Medicine, University of Iowa, Iowa City, Iowa 52242*

Abstract

We examined the effects of exercise conditioning on muscle sympathetic nerve activity (MSNA) during handgrip and post-handgrip circulatory arrest (PHG-CA). Two conditioning stimuli were studied: forearm dominance and bodybuilding. Static handgrip at 30% maximal voluntary contraction followed by PHG-CA led to a rise in MSNA smaller in dominant than in nondominant forearms (99% vs. 222%; $P < 0.02$) and in body builders than in normal volunteers (28% vs. 244%; $P < 0.01$). Separate ^{31}P NMR experiments showed no effect of dominance on forearm pH but a pH in bodybuilders higher (6.88) than in normal volunteers (6.79; $P < 0.02$) during PHG-CA.

Our second goal was to determine if factors besides attenuated [H^+] contribute to this conditioning effect. If differences in MSNA during exercise were noted at the same pH, then other mechanisms must contribute to the training effect. We measured MSNA during ischemic fatiguing handgrip. No dominance or bodybuilding effect on pH was noted. However, we noted increases in MSNA smaller in dominant than nondominant forearms (212% vs. 322%; $P < 0.02$) and in bodybuilders than in normal volunteers (161% vs. 334%; $P < 0.01$).

In summary, MSNA responses were less during exercise of conditioned limbs. Factors aside from a lessening of muscle acidosis contribute to this effect. (*J. Clin. Invest.* 1992. 89:1875–1884.) Key words: cardiovascular reflexes • exercise conditioning • microneurography • nuclear magnetic resonance • sympathetic nervous system

Introduction

Alam and Smirk (1) in 1937 demonstrated that postexercise circulatory arrest increases blood pressure. Based on these classic studies it has been suggested that during exercise, muscle ischemia contributes to an increase in sympathetic outflow through activation of a muscle metaboreflex. The skeletal muscle metabolic events that initiate and sustain this reflex response are not entirely clear, although experiments from a number of laboratories suggest an important role for skeletal muscle lactic acid production and/or reductions in muscle pH (2–6). However, it is unlikely that cellular acidosis alone is responsible for initiating this response. For example, Rotto et al. (7) have recently provided convincing evidence that cyclooxygenase blockade, which prevents prostaglandin and thromboxane synthesis, greatly attenuates group IV muscle afferent activity. Group IV afferents are thought to be part of the afferent limb of the metaboreceptor reflex arc (8). In addition, Stebbins et al. (9) have demonstrated that prostaglandins contribute to the cardiovascular reflex responses to static contraction.

Exercise conditioning has been shown to attenuate sympathetic neural responses to forearm exercise (10, 11). In part, this may be due to blunted metaboreceptor activation. Whether these attenuated neural responses are entirely due to the reduced muscle acid production seen with conditioning is not clear.

In this report, we examined the effects of conditioning on muscle sympathetic nerve activity (MSNA,[1] microneurography) and skeletal muscle pH (^{31}P NMR spectroscopy) during forearm exercise. Two strategies were employed. In the first (a within-subject comparison), we compared the effects of forearm exercise of the larger, stronger, dominant forearm to exercise of the smaller, weaker, nondominant forearm. In the second (a between-subject comparison), we compared the effects of forearm exercise in two groups of subjects, untrained controls and chronically resistance-trained subjects (bodybuilders). Two exercise protocols were utilized: the first consisted of static exercise at 30% maximal voluntary contraction (MVC) followed by 2 min of post-handgrip circulatory arrest (PHG-CA). The second employed ischemic rhythmic forearm exercise to fatigue followed by a period of PHG-CA. During these experiments we measured MSNA. In separate experiments using the same two protocols we used ^{31}P NMR techniques to investigate the effects of arm dominance and bodybuilding on forearm skeletal muscle pH.

The results of our experiments suggest that the MSNA responses to forearm exercise are attenuated by arm dominance and bodybuilding. The results further suggest that a decrease in muscle acid production is not the sole mechanism responsible for the attenuated MSNA responses.

Methods

Peroneal nerve recording experiments

Subjects. For the peroneal nerve studies 11 normal volunteers (mean age 24 yr, range 20–30) and 7 bodybuilders (mean age 27 yr, range 24–31) were studied. All were in good health and none took medica-

Address reprint requests to Dr. Sinoway, Division of Cardiology, Milton S. Hershey Medical Center, Pennsylvania State University, P.O. Box 850, Hershey, PA 17033.

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1. *Abbreviations used in this paper:* BP, blood pressure; HR, heart rate; MAP, mean arterial blood pressure; MVC, maximal voluntary contraction; MSNA, muscle sympathetic nerve activity; PHG-CA, post-handgrip circulatory arrest.

tion. All studies were approved by the appropriate institutional review boards and all subjects gave informed written consent to participate.

The bodybuilders who were studied had performed multiple upper body exercises at least 1½ h per session, four times a week, for at least 4 yr (range 4–10 yr). These exercises emphasized a large number of repetitions and moderate workloads, which were designed to increase muscle mass more than to increase strength.

In the experiments to be described we measured heart rate (HR; electrocardiogram), blood pressure (BP; automated device), respirations (pneumograph), and MSNA in the peroneal nerve using microneurography.

Microneurography technique. The details of this method have been described in detail previously (12–14). Multiunit recordings of sympathetic nerve traffic were obtained by using a tungsten electrode placed in a muscle fascicle within the peroneal nerve. The electrode has a 200-μm shaft that tapers to a 1–5-μm tip. A reference electrode was placed in the subcutaneous tissue over the fibular head and 1–3 cm from the active electrode. The neural signal was amplified 1,000 times by a preamplifier and 50–90 times by an amplifier. The resultant signal was fed through a bandpass filter (700 and 2,000 Hz). The signal was rectified and integrated to obtain a mean voltage neurogram. The neurogram was analyzed manually by counting the number of bursts and the total burst amplitude per minute. The criteria for an acceptable recording have previously been described in detail (12, 13).

Experimental protocols

Protocol 1: static handgrip exercise and PHG-CA. Each normal volunteer and body builder performed two separate forearm exercise protocols with each arm. Thus, each subject performed four bouts of exercise. In the first protocol we examined the effects of a rigorous but nonfatiguing static exercise protocol during exercise and PHG-CA. The MSNA responses during static exercise represent the combined contributions of central command, mechanosensitive, and metabosensitive muscle afferent stimulation (15). PHG-CA eliminates the contribution of central command and mechanosensitive afferents and isolates the metaboreceptor contribution (15).

HR, BP, and MSNA were recorded during 5 min of rest and 2 min of isometric handgrip at 30% MVC. A few seconds before cessation of handgrip, an upper arm cuff was inflated to suprasystolic levels thereby arresting the circulation. The subjects then ceased exercise. Circulatory arrest was continued for 2 min. This was followed by a 3-min recovery period.

Protocol 2: fatiguing rhythmic handgrip during circulatory arrest. After a 5-min recording of data, the forearm circulation was arrested. At 6 min of circulatory arrest (CA), the subject began rhythmic handgrip at 20% MVC at a rate of 30 contractions per minute and continued to exhaustion. CA was continued for an additional minute. Circulation to the forearm was restored and data were recorded for an additional 3 min. The sequence of forearms being studied was varied.

The rationale for this protocol was as follows. The forearm circulation was arrested for 6 min to exclude any influence of a difference in blood flow and oxygen stores in the conditioned vs. nonconditioned arms on MSNA responses to exercise. Exercise to fatigue during CA was performed because we postulated that this intervention would lead to similar levels of muscle pH in the dominant and nondominant forearms and in the bodybuilders and normal volunteers. This assumption was based on the fact that in the absence of oxygen, the majority of ATP generated for muscle contraction would have to come from anaerobic glycolysis. The rate-limiting step in this process is not felt to be enhanced by conditioning stimuli (16). If, under these circumstances (fatiguing handgrip during circulatory arrest with comparable decreases in forearm pH in conditioned vs. nonconditioned arms), an effect of conditioning on MSNA responses were still present, then this would suggest that mechanisms aside from cellular [H^+] production were important in mediating the influence of conditioning on metaboreceptor control of MSNA in humans. The one minute period of PHG-CA was performed to isolate the metaboreceptor contribution to MSNA responses.

^{31}P NMR spectroscopy experiments

We performed these experiments for two reasons. First, we sought to determine if our two conditioning stimuli (dominance and bodybuilding) would affect the forearm pH response to nonfatiguing static exercise (protocol 1). Second, we wished to confirm that our second protocol would cause similar levels of pH at the time of fatigue in the trained and untrained forearm groups.

Eight controls and eight bodybuilders were studied. Of the eight bodybuilders, seven contributed microneurography data to the experiments reported in the previous section. The eight normal volunteers in the NMR experiments were different from the normal volunteers included in the experiments with microneurography. Microneurography experiments in normal volunteers were performed at the University of Iowa, where facilities for NMR spectroscopy in humans were not available, and NMR experiments and microneurography experiments in body builders were performed at the Hershey Medical Center. The two groups of normal volunteers were of similar age (24±1 vs. 30±2 yr), weight (172±5 vs. 172±4 lb), and height (72±1 vs. 71±0 in).

The details of the procedures for measuring high-energy phosphate metabolites in our laboratory have been described previously (3). The ^{31}P NMR spectra were obtained with a 1.9-T, 27-cm bore superconducting magnet (Oxford Instruments, Concord, MA) interfaced to a radiofrequency transmitter/receiver (Nicolet Instrument Corp., Madison, WI). A 2.5-cm circular coil was placed on the forearm over the flexor digitorum superficialis and held in place by a piston and cylinder coil mount. Field homogeneity was optimized by adjusting the room temperature gradients to maximize the proton signal (17). The ^{31}P spectra were collected at 32.5 MHz with a 1.9-s delay between radiofrequency pulses. Spectra were obtained from the Fourier transformation of 32 transients averaged over 60 s. The concentrations of inorganic phosphate (P_i) and phosphocreatine (PCr) were determined using the areas under each respective spectral curve. Intracellular pH was calculated from the chemical shift of the P_i resonance in relation to the PCr peak (18). PH was measured during each minute of the protocols.

Statistical analysis

HR, BP, MSNA, pH, and $P_i/PCR + P_i$ were analyzed in each protocol with a two-within and one-between analysis of variance. In both protocols we tested for three main effects: dominance (dominant vs. nondominant forearm; a within-subject variable), study period (baseline vs. exercise vs. PHG-CA; a within-subject variable), and subject group (bodybuilders vs. normal volunteers; a between-subject variable). When a significant F value was found for any of the main effects, specific post-hoc analyses were made by comparing the simple effects (19).

The MSNA data were expressed as a percent change in total amplitude from baseline and then statistically analyzed. Absolute values of total amplitude were not statistically analyzed because different recording systems with different maximal amplitude scales were used for the Iowa and Hershey experiments (Iowa experiments—Gould Inc. [Cleveland, OH] recorder; Hershey experiments—Electronics for Medicine [Pleasantville, NY] recorder). In addition, total amplitude values can be influenced by electrode placement within the nerve fascicle.

Results

Table I lists the various forearm and subject characteristics in the two microneurography subject groups. There was a statistical difference in the age of the two groups; however, the mean difference was only 3 yr. The bodybuilders weighed more than the normal volunteers and had larger forearm volumes and greater MVC values. In addition, forearm volumes of the dominant arms were significantly greater than those of the nondominant arms in the normal volunteers. MVC was greater in the dominant forearms as compared to the nondominant forearms in both subject groups.

Table I. Anthropometric Characteristics of Bodybuilders and Normal Volunteers

	Age	Weight	Height	Forearm volume D	Forearm volume ND	MVC D	MVC ND
	yr	*lb*	*in*	*ml*		*kg*	
Normal volunteers (n = 11)	24±1	172±5	72±1	1,147±34	1,076±31‡	39±2	36±1‡
Bodybuilders (n = 7)	27±1*	187±5*	70±1	1,455±60*	1,435±45*	60±3	55±2*‡

Forearm volume measured by water displacement (hand volume excluded). D, dominant forearm; ND, nondominant forearm; MVC, maximal voluntary contraction. * $P \leq 0.05$ for post-hoc analysis (normal volunteers vs. body builders); ‡ $P < 0.05$ for post-hoc analysis (dominant vs. nondominant).

Protocol 1: static handgrip exercise and PHG-CA

Peroneal nerve experiments. The MAP and HR data from protocol 1 are presented in Table II. Mean arterial blood pressure (MAP) was higher at rest in the bodybuilders than in the normal volunteers, and tended to remain higher during static exercise. However, during PHG-CA the blood pressure in the two subject groups tended to be similar. Accordingly, during PHG-CA the change in MAP from baseline was much less in the bodybuilders (18-mmHg increase in the normal volunteers and an 8-mmHg increase in MAP in the bodybuilders). Overall, these findings were responsible for a subject group/exercise period statistical interaction (F = 3.3; $P < 0.01$; Table II). We noted no effect of dominance on the MAP response to exercise. There was no effect of subject group or forearm dominance on the heart rate response observed during protocol 1.

Several observations regarding the MSNA response to protocol 1 should be noted. First, the bodybuilders had an attenuated rise in MSNA (subject effect: $F = 8.1$, $P < 0.01$, Fig. 1 *A*). Post-hoc analysis showed differences between the bodybuilder and normal volunteer groups during the 2nd min of static exercise and during the 2 min of PHG-CA (Fig. 1 *A*).

In addition, we noted a dominance effect, with less of an increase in MSNA during dominant than during nondominant forearm exercise. Post-hoc analysis demonstrated statistical differences between the groups during the 2 min of PHG-CA (Fig. 1 *B*). 9 of 11 normal volunteers and 5 of 7 bodybuilders had less of an increase in MSNA during the PHG-CA that followed dominant forearm static exercise (normal volunteers—nondominant forearm, 357% increase; dominant forearm, 186% increase; bodybuilders—nondominant forearm, 47% increase; dominant forearm, 16% increase in MSNA during PHG-CA). The effects of dominance appeared to be greater in the normal volunteers in that a subject/handedness interaction was present (F = 4.3; P = 0.05). The absolute burst counts and bursts per 100 heart beats for this protocol are shown in Table III.

NMR experiments. We observed a subject effect for pH during protocol 1. Specifically, pH responses were attenuated in the bodybuilders (F = 4.7; $P < 0.05$, Fig. 2 *A*) with significant differences between the two subject groups during the 2 min of static exercise (bodybuilders, 6.99; normal volunteers, 6.89; $P < 0.02$) and during the 2 min of PHG-CA (minute 1 of PHG-CA: bodybuilders, 6.88; normal volunteers, 6.79; $P < 0.02$; minute 2 of PHG-CA: bodybuilders, 6.86; normal volunteers, 6.78; $P < 0.04$). We observed no effect of dominance on the pH response to static exercise during this protocol (Fig. 2 *B*). The $P_i/PCr + P_i$ data for this experiment are shown in Table IV.

Table II. HR and MAP Data from Protocol 1

	Base	Grip 1	Grip 2	PHG-CA 1	PHG-CA 2	Recovery	Statistical main effects and interactions
HR (*beats/min*)							
Normal volunteers	56±1	66±2	73±2	61±2	58±1	57±1	Exercise F = 24.7; $P < 0.01$
Bodybuilders	59±2	68±3	70±3	60±3	59±2	60±2	Dominance F = 2.1; P = NS
Dominant	58±2	67±2	72±2	62±3	59±1	59±1	Subject group F = 0.03; P = NS
Nondominant	57±2	66±2	71±3	60±2	58±2	57±2	
MAP (*mmHg*)							
Normal volunteers	83±1	88±2	101±3	101±3	101±3	88±2	Exercise F = 25.8; $P < 0.01$
Bodybuilders	93±2*	99±3*	107±3	101±3	101±3	91±2	Dominance F = 0.3; P = NS
Dominant	89±2	95±3	104±3	101±2	99±2	89±2	Subject group F = 2.5; P = NS
Nondominant	85±2*	90±2	103±4	102±3	102±3	89±2	Dominance · exercise F = 2.1; $P < 0.08$
							Subject · exercise F = 3.3; $P < 0.01$

Data presented from 11 normal volunteers and 7 bodybuilders. Dominant and nondominant data represent values from both bodybuilders and normal volunteers (i.e., n = 18 for each observation). Grip represents 1 min of static forearm exercise. PHG-CA, post-handgrip circulatory arrest. * $P < 0.05$ for post-hoc analysis (dominant vs. nondominant or normal volunteers vs. bodybuilders).

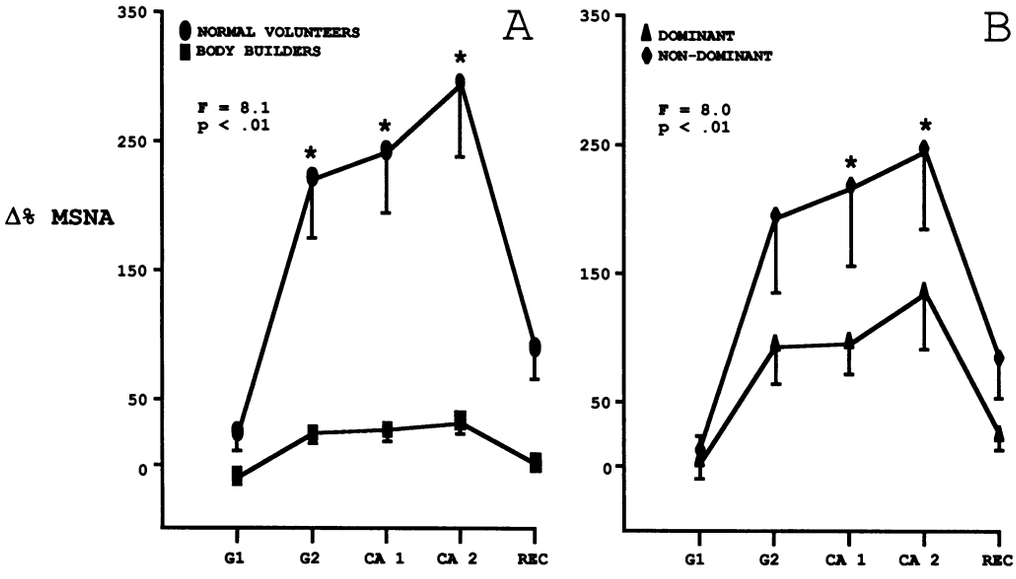

Figure 1. Percent change in the total amplitude of MSNA (Δ% MSNA) for (*A*) normal volunteers (n = 11) vs. bodybuilders (n = 7) and (*B*) dominant vs. nondominant forearms (n = 18) during protocol 1 (see text). *G1* and *G2*, 1st and 2nd min of static exercise; *CA1* and *CA2*, 1st and 2nd min of post-handgrip circulatory arrest; *REC*, recovery. F value in *A* is for the subject effect (bodybuilders vs. normal volunteers) and in *B* for dominance effect (dominant vs. nondominant forearms). Of note, a subject/dominance interaction was noted (F = 4.3; P < 0.05, not shown above) suggesting the dominance effect was less in the bodybuilders. Bars below data points represent standard error. *Statistical differences between comparable points (simple effects method).

Protocol 2: fatiguing rhythmic handgrip during circulatory arrest

Peroneal nerve experiments. The HR and MAP data obtained during this protocol are shown in Table V. We noted no significant effect of dominance or subject group on HR or MAP during this protocol. The time to fatigue during ischemic contractions were similar in the dominant and nondominant forearms of the bodybuilders and controls (bodybuilders—dominant forearm, 3.6±0.6 min; nondominant forearm, 3.0±0.5 min; normal volunteers—dominant forearm, 3.1±0.2 min; nondominant forearm, 3.4±0.2 min).

There are several findings regarding the MSNA data obtained during the second protocol that should be mentioned. First, there was a subject effect (F = 6.2; P < 0.03, Fig. 3 *A*). Post-hoc analysis demonstrated that MSNA was much higher during the last minute of rhythmic ischemic exercise in the normal volunteers (bodybuilders, 105% increase; normal volunteers, 380% increase; Fig. 3 *A*). The increase in MSNA was also higher during the PHG-CA period in the normal volunteers than in the bodybuilders (bodybuilders, 161% increase; normal volunteers, 334% increase). We also noted a dominance effect such that the increase in MSNA was less during protocol 2 when the dominant forearms were exercised (F = 9.6; P < 0.01). Post-hoc analysis demonstrated that the percent increase in MSNA was less in the dominant forearm group than in the nondominant forearm group during the last minute of ischemic exercise and during the minute of PHG-CA. Representative neurograms during rest and PHG-CA in a bodybuilder and a normal volunteer are seen in Fig. 4. The absolute values for MSNA bursts and bursts per 100 heart beats for this protocol are shown in Table VI.

Table III. Sympathetic Burst Counts and Bursts per 100 Heart Beats from Protocol 1

	Base	Grip 1	Grip 2	PHG-CA 1	PHG-CA 2	Recovery
Bursts (*bursts/min*)						
Normal volunteers	18±2	21±3	35±3	31±2	36±3	24±2
Bodybuilders	31±0	27±3	34±3	32±3	35±3	31±3
Dominant	23±3	23±3	32±3	29±2	35±3	26±3
Nondominant	23±3	23±3	38±3	34±2	36±3	28±2
Bursts per 100 heart beats						
Normal volunteers	33±4	33±4	50±4	50±3	61±4	43±4
Bodybuilders	51±5	41±6	47±4	53±5	56±5	50±5
Dominant	38±5	34±5	44±4	47±4	59±5	45±5
Nondominant	41±5	38±5	53±4	55±4	60±4	46±4

Data from 11 normal volunteers and 7 bodybuilders. Dominant and nondominant data represent values from both bodybuilders and normal volunteers (i.e., n = 18 for each observation). Explanations of headings as in Table II. For statistics of MSNA data, see Fig. 1.

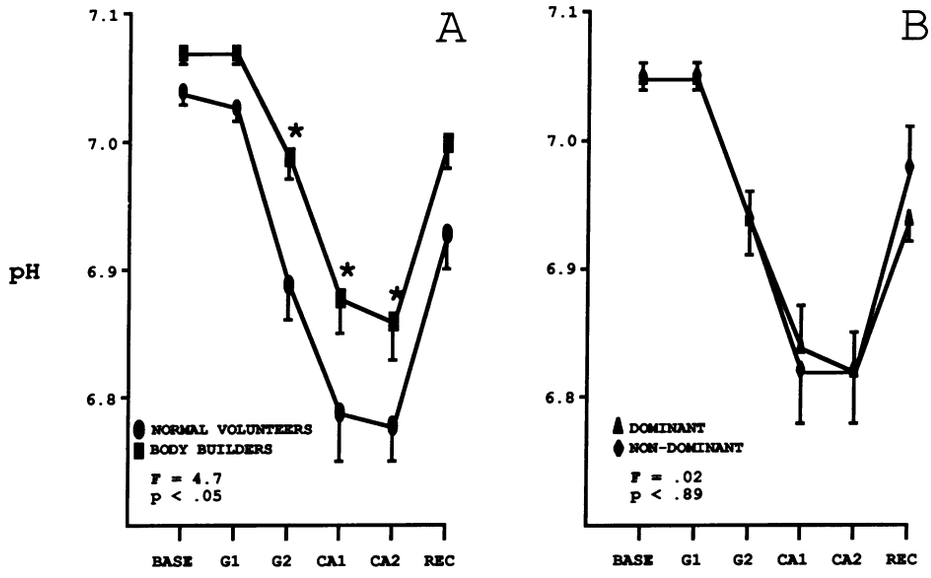

Figure 2. Forearm muscle pH during static handgrip and PHG-CA (protocol 1). Abbreviations as in Fig. 1. (*A*) In the bodybuilders forearm pH is higher during sustained handgrip and post-handgrip circulatory arrest than it is in the normal volunteers. (*B*) There is no dominance effect (n = 8 for normal volunteers and bodybuilders).

NMR experiments. In protocol 2 we observed a very large reduction in pH during the last minute of fatiguing rhythmic handgrip and during PHG-CA with values in the 6.3–6.4 range. We observed no subject or arm dominance effect for pH (Fig. 5). Table VII lists the $P_i/PCr + P_i$ data for this experiment.

Lower body negative pressure experiments

In five normal volunteers and four bodybuilders we compared MSNA responses to −30 mmHg of lower body negative pressure. This is a stimulus that disengages both high and low pressure baroreceptors and accordingly causes sympathoexcitation. We observed a 131% increase in MSNA in the normal volunteers and an 86% increase in the bodybuilders (NS by t test).

Discussion

In this report we have demonstrated that both arm dominance and bodybuilding attenuate MSNA responses during exercise. The NMR data suggest that a portion of the subject effect related to bodybuilding may be due to an attenuated muscle acidosis during exercise, since the fall in forearm pH during the sustained handgrip at 30% MVC was less in the bodybuilders than in the normal volunteers. However, arm dominance had no effect on forearm pH during sustained handgrip at 30% MVC, yet MSNA responses rose less with exercise of the dominant forearm than of the nondominant forearm. In the second protocol we observed similar levels of cellular acidosis during fatiguing ischemic handgrip in the dominant and nondominant forearms and in the bodybuilders and normal volunteers. Despite this, we observed an attenuated effect of arm dominance and bodybuilding on MSNA responses. Thus, training-induced reductions in cellular acid production cannot be the sole explanation for the smaller increases in MSNA seen in response to exercise with arm dominance and bodybuilding. This discussion section will focus on the study design and rationale, potential limitations of our results, and the potential mechanisms that may explain these findings.

Study design and rationale. Lactic acid production and/or reductions in skeletal muscle pH have been demonstrated to be potent stimulants of skeletal muscle metaboreceptors (2–6, 20, 21). In this study our goal was to determine if conditioning stimuli of different intensities would attenuate metaboreceptor responses. The stimuli chosen were forearm dominance and bodybuilding. As compared to the nondominant forearm, the dominant forearm has a greater endurance capacity during exercise (10). Moreover, it has been suggested that during the same level of forearm work the dominant forearm will become less acidic than the nondominant forearm (22).

Bodybuilders exercise to increase muscle mass. In general, these athletes perform moderate to high workload, high repeti-

Table IV. $P_i/PCr + P_i$ Data from NMR Experiments for Protocol 1

	Base	Grip 1	Grip 2	PHG-CA 1	PHG-CA 2	Recovery	Statistical main effects and interactions
Normal volunteers	0.09±0.01	0.30±0.02	0.40±0.03	0.42±0.03	0.43±0.03	0.14±0.01	Exercise F = 128.9; $P < 0.01$
Bodybuilders	0.10±0.01	0.29±0.03	0.37±0.03	0.38±0.04	0.39±0.03	0.12±0.01	Dominance F = 0.1; P = NS
Dominant	0.09±0.01	0.32±0.03	0.39±0.03	0.41±0.03	0.41±0.02	0.13±0.01	Subject group F = 0.4; P = NS
Nondominant	0.11±0.01	0.27±0.02	0.38±0.03	0.40±0.04	0.41±0.04	0.14±0.01	Subject · dominance F = 4.8; P = 0.05
							Subject · dominance · exercise F = 2.9; $P < 0.02$

Data from eight normal volunteers and eight bodybuilders. Dominant and nondominant data represent values from both bodybuilders and normal volunteers (i.e., n = 16 for each observation). Explanations of headings as in Table II.

Table V. HR and MAP Data from Protocol 2

	Base	CA	Last min of grip	PHG-CA	Recovery	Statistical main effects and interactions
HR (*beats/min*)						
Normal volunteers	54±1	56±1	76±1	63±1	56±1	Exercise $F = 110.0$; $P < 0.01$
Bodybuilders	59±2	61±2	82±2*	67±2	61±2	Dominance $F = 0.7$; P = NS
Dominant	56±2	58±2	78±2	63±2	58±2	Subject group $F = 3.7$; P = NS
Nondominant	55±1	58±2	79±2	66±2	58±2	
MAP (*mmHg*)						
Normal volunteers	83±2	83±2	114±4	109±3	91±3	Exercise $F = 83.3$; $P < 0.01$
Bodybuilders	89±2	90±2	120±3	117±2	94±3	Dominance $F = 1.0$; P = NS
Dominant	87±2	87±2	117±4	113±3	94±3	Subject group $F = 1.9$; P = NS
Nondominant	85±2	85±2	116±4	112±3	91±3	

Data presented from 11 normal volunteers and 7 bodybuilders. Dominant and nondominant data represent values from both bodybuilders and normal volunteers (i.e., $n = 18$ for each observation). CA, mean of 6 min of circulatory arrest; Grip, last minute of fatiguing rhythmic handgrip exercise during CA; PHG-CA, post-handgrip circulatory arrest. * $P < 0.05$ for post-hoc analysis (dominant vs. nondominant or normal volunteers vs. bodybuilders).

tion exercise with a number of different muscle groups. This is contrasted with power lifters who perform extremely high workload, low repetition exercise. Accordingly, we speculated that bodybuilders as opposed to power lifters would serve as a model of chronic high-level symmetrical endurance training (23, 24).

We chose 2 min of 30% MVC followed by a period of PHG-CA as our first paradigm because it is not usually fatiguing yet it increases MSNA (4, 13) and leads to significant reductions in forearm skeletal muscle pH (3, 4). Moreover, the period of PHG-CA allows one to isolate the MSNA response due to metaboreceptor stimulation.

The results from protocol 1 suggest that the markedly attenuated MSNA responses seen in the bodybuilders were perhaps in part mediated by a conditioning-induced reduction in muscle acid production inasmuch as the fall in forearm pH was less in the bodybuilders than in the control subjects. However, in protocol 1 we noted similar forearm pH responses in the dominant and nondominant forearms. In separate experiments, the MSNA responses were less during dominant forearm exercise. This suggested that influence(s) aside from the production of acid may affect the magnitude of the MSNA response during isolated metaboreceptor stimulation.

In addition, the effects of dominance on MSNA and pH in protocol 1 excluded a training-induced alteration in the set point for this stimulus/response relationship. If there had been an altered set point, then the pH at which MSNA began to rise during exercise in the trained forearm (in this case the dominant forearm) would have been different (i.e., lower) than that for the untrained forearm (the nondominant forearm). However, it can easily be seen that the pH curves in Fig. 2 *B* are superimposable. In Fig. 1 *B* the time course for the MSNA response for the dominant and nondominant forearm groups are very similar in appearance. If there were a major shift in the

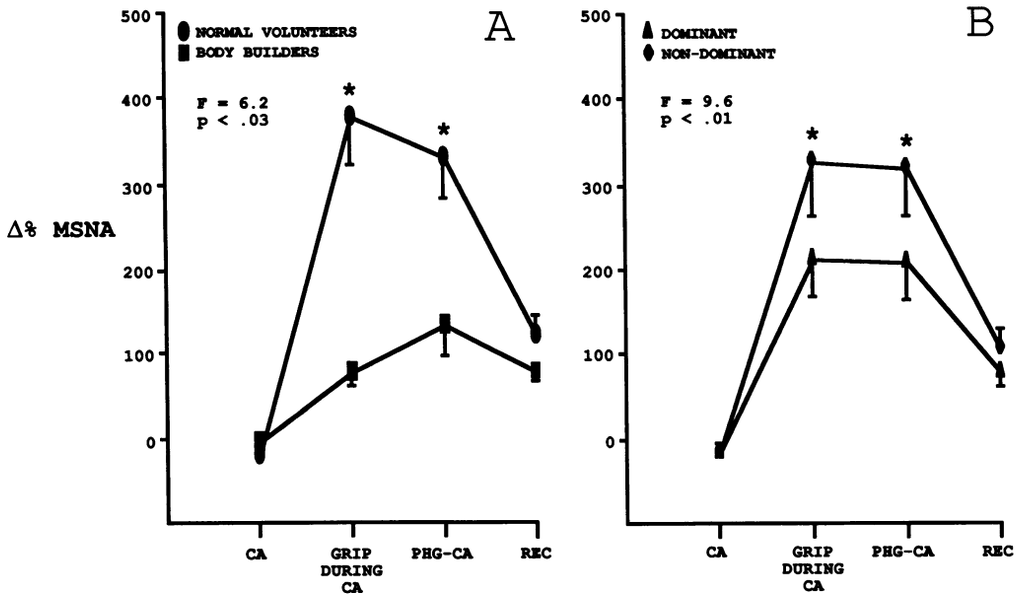

Figure 3. Percent change in MSNA during ischemic fatiguing handgrip and PHG-CA (protocol 2). *CA,* 6-min period of forearm circulatory arrest preceding ischemic exercise; GRIP, last minute of fatiguing rhythmic handgrip during CA. PHG-CA is data from 1 min of circulatory arrest after ischemic exercise. Of note, a subject/dominance interaction was noted ($F = 6.5$; $P < 0.01$) suggesting that the effects of dominance were less in the bodybuilders.

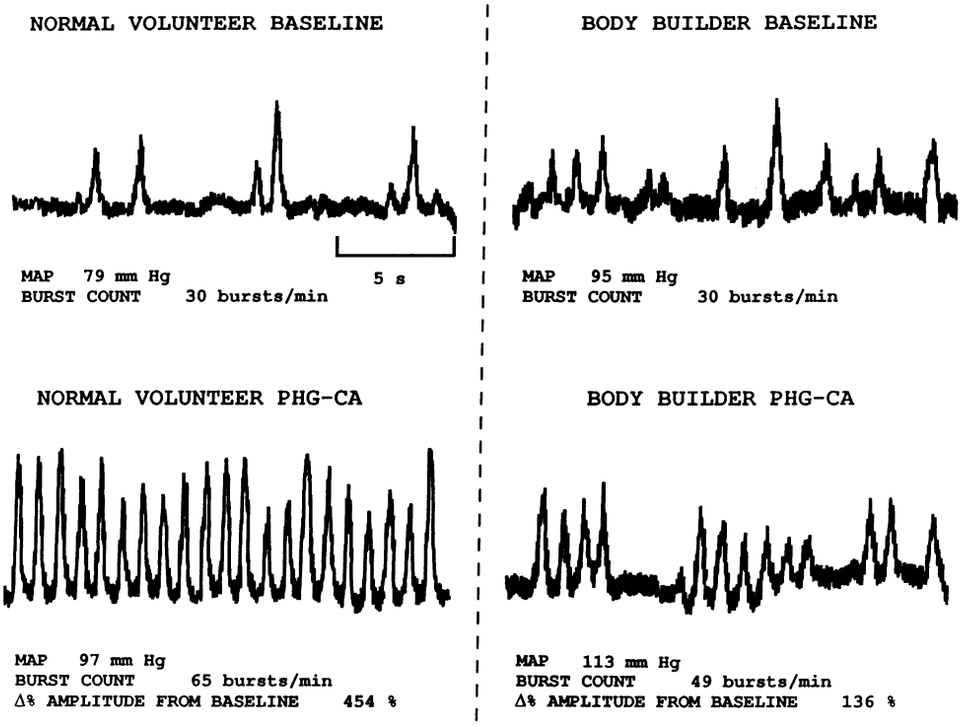

Figure 4. Representative integrated neurograms from protocol 2 (nondominant forearm). The normal volunteer is presented on the left and the bodybuilder on the right. Baseline data are shown in the upper two panels and PHG-CA data after fatiguing rhythmic handgrip during circulatory arrest are shown in the lower panels. Of note, the percent increase in MSNA was much less in the bodybuilder than in the normal volunteer.

set point, then we would have expected a delay in the onset of the increase in MSNA in the dominant forearm as compared to the nondominant forearm. This was not the case. Accordingly, we believe this data supports the concept that the set point was not altered.

Potential explanations for this non–pH-mediated effect included: (*a*) conditioning-induced changes in flow and/or oxygen delivery, (*b*) an effect of conditioning on forearm oxygen stores, (*c*) a primary effect of forearm dominance such that the central nervous system interprets metaboreceptor signals differently from dominant and nondominant forearms, or (*d*) a conditioning-induced desensitization or adaptation of the muscle metaboreceptor such that it responds less to a given amount of acid.

To address these possible mechanisms we performed the second protocol. We arrested the circulation for 6 min before initiating forearm exercise. This period of occlusion was chosen to eliminate blood flow and also to maximally reduce forearm oxygen stores (25). We reasoned that this would minimize any training-induced influences of vascularity and/or oxygen stores on the MSNA and forearm pH responses to exercise. In addition, we had the subjects perform exercise until fatigue in the absence of flow and oxygen, thereby limiting fatty acid delivery to muscle and increasing muscle cell reliance on anaerobic glycolysis. We speculated that in this protocol the minimal forearm pH would be similar in the dominant and nondominant forearms and in normal volunteers and bodybuilders. This hypothesis was based on the concept that both

Table VI. Sympathetic Burst Counts and Bursts per 100 Heart Beats from Protocol 2

	Base	CA	Last min of grip	PHG-CA	Recovery
Bursts (*bursts/min*)					
Normal volunteers	22±2	18±2	52±2	42±2	32±2
Bodybuilders	28±3	26±3	46±5	42±3	34±3
Dominant	24±3	21±3	47±3	40±3	32±2
Nondominant	24±3	21±3	53±3	45±2	34±2
Bursts per 100 heart beats					
Normal volunteers	40±4	32±4	71±3	66±4	57±3
Bodybuilders	49±5	44±5	56±5	63±4	56±5
Dominant	43±5	36±5	63±4	61±4	54±4
Nondominant	44±5	38±5	67±4	69±3	59±4

Data from 11 normal volunteers and 7 bodybuilders. Dominant and nondominant data represent values from both bodybuilders and normal volunteers (i.e., $n = 18$ for each observation). Explanations of headings as in Table V. For statistics of MSNA data, see Fig. 3.

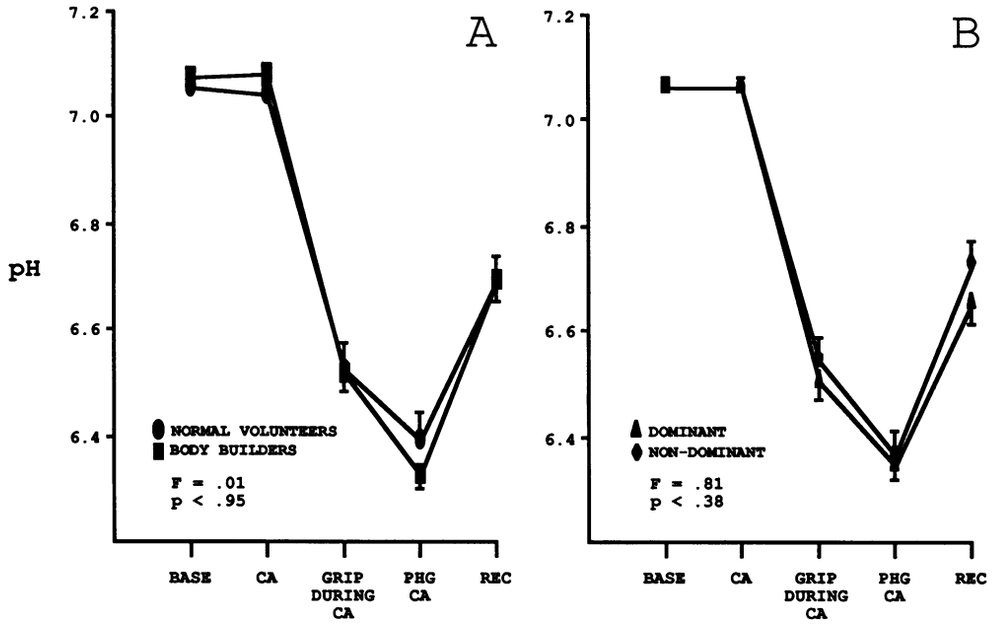

Figure 5. Forearm muscle pH during ischemic fatiguing handgrip exercise and PHG-CA (protocol 2). Abbreviations as in Fig. 3. Of note, no effect of body building (*A*) or dominance (*B*) is demonstrated.

the β oxidation of free fatty acids and the aerobic utilization of muscle glycogen would be affected by conditioning, whereas anaerobic glycolysis would not (16). Accordingly, if fatiguing forearm exercise performed during circulatory arrest still was associated with smaller increases in MSNA in the bodybuilders, then mechanisms aside from cellular acidosis, blood flow, oxygen stores, and forearm dominance must be important in mediating muscle metaboreceptor responses. The results of protocol 2 suggest that fatiguing exercise performed in the absence of blood flow leads to similar levels of cellular acidosis in trained and untrained muscle. Despite this, exercising trained muscle activated the sympathetic nervous system less than did untrained muscle. These results suggest that factors aside from muscle acidosis contribute to the conditioning-induced attenuation of MSNA responses to exercise.

Potential mechanisms. There are a number of potential mechanisms that could explain the conditioning-induced attenuation of MSNA responses to fatiguing ischemic exercise. First, it is possible that conditioning modifies group III and IV discharge properties such that they respond less to a given amount of produced lactic acid. Specifically, it has been suggested that muscles composed of slow twitch fibers do not generate a pressor response when stimulated to contract (26). However, this issue remains unsettled since others have shown in a cat model that the slow twitch soleus muscle will generate a pressor response if made to contract maximally (27).

Although we know of no direct evidence that conditioning modifies afferent nerve fibers, it has been demonstrated that exercise conditioning causes both biochemical and physiologic changes in motor neurons that innervate trained skeletal muscle (28). It is possible that both arm dominance and bodybuilding cause biochemical and physiologic changes in the group III and IV fibers. These changes could theoretically serve to reduce afferent fiber responsiveness to a given amount of lactic acid.

We considered the possibility that the attenuated MSNA responses during forearm exercise and postexercise ischemia seen in the bodybuilders were due to a generalized reduction in sympathetic responsiveness. For this reason, we performed lower body negative pressure experiments in four bodybuilders and five normal volunteers. We noted an 86% rise in MSNA in the bodybuilders and a 131% increase in the normal volunteers. This is quite different from the situation seen during the exercise protocols, where the magnitude of the increase in MSNA in the normal volunteers was dramatically larger than that seen in the bodybuilders. Thus, our LBNP studies suggest it is unlikely that a generalized impairment of sympathoexcitation is present in the bodybuilders.

Another possibility is that conditioning reduces metabore-

Table VII. $P_i/PCr + P_i$ Data from NMR Experiments for Protocol 2

	Base	CA	Last min of grip	PHG-CA	Recovery	Statistical main effects and interactions
Normal volunteers	0.08±0.01	0.10±0.01	0.67±0.02	0.68±0.17	0.20±0.02	Exercise $F = 506.2$; $P < 0.01$
Bodybuilders	0.10±0.01	0.11±0.01	0.74±0.04*	0.79±0.03*	0.19±0.02	Dominance $F = 0.6$; P = NS
Dominant	0.09±0.01	0.10±0.01	0.74±0.03	0.75±0.03	0.19±0.02	Subject group $F = 3.6$; P = NS
Nondominant	0.10±0.01	0.12±0.01	0.68±0.04	0.72±0.03	0.20±0.01	Subject · exercise $F = 3.0$; $P < 0.03$

Data from eight normal volunteers and eight bodybuilders. Dominant and nondominant data represent values from both bodybuilders and normal volunteers (i.e., $n = 16$ for each observation). Explanations of headings as in Table V.

ceptor responses through a prostaglandin-mediated process. Recent experiments by Rotto et al. (7) suggest that cyclooxygenase blockade attenuates group IV muscle afferent responses to static contractions. From these results the authors (7) have concluded that "prostaglandins and/or thromboxanes are needed for the full expression of both the mechanical and metabolic responses of group IV muscle afferents to static contraction." It is possible that conditioned muscle releases less prostaglandin or prostaglandin metabolites than nontrained muscle and less sensitization of the muscle metaboreceptor occurs. Further studies will be necessary to determine whether prostaglandin release during exercise is reduced by exercise conditioning.

Finally, it should be stated that little is known about the transductive properties of group III and IV afferents. Therefore, precise statements regarding the mechanisms responsible for their activation must await further study.

Potential limitations. First, MSNA responses reflect changes in sympathetic outflow to only one vascular bed. Accordingly, the influence of conditioning stimuli on sympathetic outflow to other important end organs cannot be extrapolated from our findings. Second, arm dominance and bodybuilding are two highly specific training stimuli. Third, ^{31}P NMR reflects pH changes in a very small muscle mass. Thus, whether the conditioning effects we have noted can be generalized to other training protocols and to other skeletal muscles remains to be determined.

The NMR measurements used in these studies measure only intracellular pH. This is a potential concern since the group IV nerve endings that act as metabosensors are likely to terminate near capillaries and venules in the skeletal muscle interstitium (29). However, prior studies in animals using microelectrodes have shown that with exercise interstitial pH falls as exercise continues (30). This fall parallels changes in muscle venous $[H^+]$. Because the source of the hydrogen ion in the interstitium and veins must be the skeletal muscle cell, it is likely that the cellular pH is a reasonable index of interstitial pH.

Along similar lines, it could be argued that the differences in pH between the bodybuilders and normal volunteers during protocol 1 are too small to be of physiologic significance. However, if the data were to be expressed as $[H^+]$, then we would note a 78% increase in $[H^+]$ in the normal volunteers during exercise whereas the bodybuilders increase $[H^+]$ by only 55%. This degree of attenuation is likely to be significant. Our laboratory has recently shown in humans that when dichloroacetate infusions were administered, there was a 29% reduction in the forearm venous $[H^+]$ response to exercise. This was associated with a 50% fall in the MSNA response to static exercise.

Our findings of a dominance effect on MSNA responses during isometric nonfatiguing exercise are qualitatively different from those previously reported by Seals (31), who found no differences in MSNA responses when comparing left and right forearms during static exercise. However, the influence of forearm dominance on MSNA responses was not directly addressed and the number of subjects who were left- or right-handed was not mentioned. Furthermore, the PHG-CA maneuver was not performed because an evaluation of metaboreceptor responses was not a goal of this prior study.

The attenuated BP responses during nonfatiguing isometric handgrip exercise in the bodybuilders are qualitatively different from the results from a prior study by Longhurst et al. (32) in which BP responses during static forearm exercise were not attenuated as compared to control subjects. Part of the difference in results may be due to differences in the protocols used in that Longhurst et al. (32) used 40% MVC and we used 30% MVC.

Conclusion. These studies suggest that the conditioning influence of arm dominance and bodybuilding attenuate reflex increases in sympathetic nerve activity during exercise and postexercise ischemia. Our studies suggest that factors aside from greater vascularity and less acid production contribute to this effect.

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