Muscle sympathetic nerve responses to physiological changes in prostaglandin production in humans

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Muscle sympathetic nerve responses to physiological changes in prostaglandin production in humans. J Appl Physiol 90: 624–629, 2001.—Previous studies suggest that prostaglandins may contribute to exercise-induced increases in muscle sympathetic nerve activity (MSNA). To test this hypothesis, MSNA was measured at rest and during exercise before and after oral administration of ketoprofen, a cyclooxygenase inhibitor, or placebo. Twenty-one subjects completed two bouts of graded dynamic and isometric handgrip to fatigue. Each exercise bout was followed by 2 min of postexercise muscle ischemia. The second exercise bouts were performed after 60 min of rest in which 11 subjects were given ketoprofen (300 mg) and 10 subjects received a placebo. Ketoprofen significantly lowered plasma thromboxane B₂ in the drug group (from 36 ± 6 to 22 ± 3 pg/ml, P < 0.04), whereas thromboxane B₂ in the placebo group increased from 40 ± 5 to 61 ± 9 pg/ml from trial 1 to trial 2 (P < 0.008). Ketoprofen and placebo did not change sympathetic and cardiovascular responses to dynamic handgrip, isometric handgrip, and postexercise muscle ischemia. There was no relationship between thromboxane B₂ concentrations and MSNA or arterial pressure responses during both exercise modes. The data indicate that physiological increases or decreases in prostaglandins do not alter exercise-induced increases in MSNA and arterial pressure in humans. These findings suggest that contraction-induced metabolites other than prostaglandins mediate MSNA responses to exercise in humans.

EXERCISE ELICITS INCREASES in muscle sympathetic nerve activity (MSNA). It is believed that reflexes originating from the exercising muscle are primarily responsible for this response. Animal studies indicate that engagement of both group III and IV muscle afferents mediate this reflex (14). These muscle afferents are sensitive to mechanical (1, 7, 11), chemical (11, 13, 19), and thermal (8, 12, 15, 16) stimuli, all of which are produced during exercise. The major stimulus for increases in MSNA during exercise in humans is believed to be the activation of muscle afferents via metabolic by-products produced by the contracting muscle (13, 17, 24).

Studies have indicated a number of possible metabolites that may stimulate these muscle afferents during exercise (see Ref. 10). Animal studies have demonstrated that arachidonic acid and the metabolites of the cyclooxygenase pathway (i.e., prostaglandins) stimulate muscle afferents and can alter the pressor response to muscle contraction (18, 21, 23). However, studies examining the effect of prostaglandins on cardiovascular responses to exercise in humans are limited and equivocal. Davy et al. (3) showed that indomethacin, a cyclooxygenase inhibitor that blocks the synthesis of prostaglandins, had no effect on arterial pressure responses to isometric handgrip (IHG). In contrast, Fontana et al. (4) reported that intravenous infusion of ketoprofen, also a cyclooxygenase inhibitor, attenuated the pressor response to isometric handgrip. Presently, no studies have examined MSNA responses to changes in prostaglandin production. MSNA should be a more direct marker than arterial pressure on the effect that prostaglandins have on muscle afferents.

Because of the limited data on this topic and contrasting results, and because these studies have only examined decreases in prostaglandin production without directly measuring MSNA, the purpose of the present study was to determine whether both physiological increases and decreases in prostaglandins affect exercising MSNA and arterial pressure. We hypothesized that if prostaglandins contribute to MSNA and arterial pressure responses during exercise, then increases in prostaglandins would increase MSNA and arterial pressure, whereas decreases in prostaglandins would attenuate MSNA and arterial pressure. To test this hypothesis, MSNA and arterial pressure responses were determined during both isometric (IHG) and dynamic (DHG) handgrip and postexercise muscle ischemia (PEMI) before and after ingestion of ketoprofen or placebo in humans. Ketoprofen, a cyclooxygenase inhibitor, reduces prostaglandin production, whereas previous exercise in the placebo trial would elicit increases in prostaglandins. The results of this

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study indicate that prostaglandins over a large physiological range do not mediate increases in MSNA during exercise in humans.

METHODS

Subjects. Twenty-one healthy subjects (14 men, 7 women; 19–33 yr), who were normotensive and not taking any medication, volunteered to participate in the study. The testing protocols were verbally explained to each subject, and written, informed consent was obtained from all participants. The study was approved by the Institutional Review Board of the Pennsylvania State University College of Medicine.

Experimental design. All subjects were tested in the supine position, and an intravenous catheter was inserted in the antecubital fossa. A baseline blood sample was then drawn for determination of thromboxane B₂ levels (TxB₂). Two bouts of forearm exercise, IHG followed by DHG, were performed to fatigue followed by PEMI at the conclusion of each exercise bout (trial 1). A 10- to 15-min rest period separated the exercise bouts, which were performed with the opposite forearm. These exercise bouts were then repeated after 60 min of rest (trial 2) and were completed within ~40 min. At the beginning of this rest period, each subject was administered either 300 mg of ketoprofen (n = 11) or a placebo tablet (n = 10) in a randomized and blinded fashion. After 60 min had elapsed, another blood sample was drawn to redetermine TxB₂. During all exercise bouts, MSNA, mean arterial pressure (MAP), and heart rate were measured. Additionally, at 1-min intervals during exercise, the subjects were asked to give ratings of perceived effort determined by the 15-point Borg scale (2). Exercise increases prostaglandin production; thus, during the placebo trial, prostaglandins would be elevated during the second exercise bout, whereas ketoprofen, an effective cyclooxygenase inhibitor, was used to decrease prostaglandin production. Thus the experimental paradigm was designed to elicit a wide range of prostaglandin levels in the body.

IHG exercise was performed to fatigue using 30% of the subject’s previously determined maximum voluntary contraction. DHG was performed at a rate of 30 contractions/min using the contralateral arm. Subjects began the protocol by squeezing the handgrip apparatus with no weight. Except for the first increment of 1.5 kg, weight was increased 1.1 kg for each successive minute until fatigue. A metronome was set at 1 pulse/s to assist the subjects in maintaining a constant gripping frequency. Each work rate lasted 1 min and was followed by 15 s of rest. At the end of each bout, subjects were asked to give ratings of perceived effort.

Five seconds before the end of the exercise bouts, a pneumatic cuff was inflated to suprasystolic pressure (240 mmHg) around the exercising arm for 2 min to elicit PEMI. Because muscle contraction ceases and blood is trapped in the exercising forearm during PEMI, it is believed that this maneuver isolates the muscle metaboreflex (i.e., stimulation of chemically sensitive muscle afferents).

Ketoprofen is a potent cyclooxygenase inhibitor that is rapidly absorbed after oral administration. Maximal concentrations in plasma are usually obtained within 60 min (30–120 min) with a half-life in plasma of ~2 h. Because food intake can alter absorption rate of ketoprofen, no subjects ate for 2–3 h prior to the administration of the drug.

Measurements. Multifiber MSNA recordings were obtained by using a tungsten microelectrode inserted into the peroneal nerve located at the popliteal fossa or head of the fibula of a resting leg. A reference electrode was placed subcutaneously 2–3 cm from the recording electrode. The recording electrode was then adjusted until a site was located where spontaneously occurring muscle sympathetic bursts were observed. Criteria for an acceptable MSNA recording site have been previously reported (9). The nerve signal was filtered with a bandwidth of 700–2,000 Hz and passed through a resistance capacitance integrating network with a time constant of 0.1 s to obtain a mean voltage display of the nerve recording.

Arterial blood pressure and heart rate were continuously measured by use of a Finapres blood pressure monitoring unit (Ohmeda, Englewood, CO) on the contralateral, nonexercising hand.

At the start of the testing procedures and 60 min after administration of the ketoprofen or placebo, a venous blood sample was collected through a catheter inserted in the brachial vein. The blood sample was stored in ice and subsequently spun for plasma. Plasma TxB₂ was used as an indicator of cyclooxygenase activity and prostaglandin production. Thromboxane A₂, a product of arachidonic acid metabolism, is very unstable and rapidly undergoes conversion to TxB₂. TxB₂ was measured by RIA in duplicate. Preparation of samples involved ethyl acetate extraction. RIA employed tritiated prostaglandin and specific rabbit antibody (PreSeptive Biosystems, Framingham, MA). All samples were analyzed together after the completion of all tests. The lower limit of detection is 4.7 pg/ml, and the intra-assay coefficient of variation was 5%.

Data analysis. All measurements were divided into 30-s intervals for analysis purposes. Bursts of sympathetic activity were identified by inspection of the mean voltageogram. Total MSNA was determined by summing the amplitudes of the bursts. Statistical analysis of collected data was performed by using two within-factors (time and exercise trial) repeated-measures ANOVA for the placebo and drug groups. A significance level of P < 0.05 was employed for all tests. Because of the variance in performance time between subjects for IHG and DHG, the responses were normalized as a function of time to fatigue (endurance time) (i.e., 25, 50, 75, and 100%). The relationship between TxB₂ levels and either MSNA or MAP was assessed by a linear regression. All values are presented as means ± SE.

RESULTS

Preexercise plasma TxB₂ levels during the ketoprofen and placebo trials are presented in Fig. 1. During

![Fig. 1. Plasma thromboxane B₂ concentrations before exercise trials 1 and 2 in the placebo and ketoprofen groups. Ketoprofen significantly reduced thromboxane B₂ concentrations, whereas thromboxane B₂ was significantly increased in the placebo group. *Significantly different from trial 1, P < 0.05. †Significantly different between groups, P < 0.05.](http://jap.physiology.org/348/20/58/348x115 to 540x262)
the ketoprofen trial, plasma levels decreased from 36 ± 6 to 22 ± 3 pg/ml (P < 0.04) from exercise trial 1 to trial 2. Conversely, the TxB2 in the placebo group rose significantly from trial 1 to trial 2 (40 ± 5 to 61 ± 9 pg/ml, P < 0.008). The range in TxB2 elicited by the experimental design was 13–100 pg/ml.

Baseline values for MSNA (bursts/30 s), MAP, and heart rate did not differ significantly between trials 1 and 2 for both the ketoprofen and placebo groups during DHG and IHG exercise protocols with the exception of MAP being greater and heart rate lower during trial 2 of DHG in the placebo group (Table 1).

IHG. MSNA responses to IHG were not significantly different between exercise trials in the ketoprofen and placebo groups. MSNA increased by 14 ± 4 and 17 ± 4 bursts/30 s from baseline during trials 1 and 2, respectively, for the ketoprofen group (Fig. 2). Total MSNA increased 287 ± 38 and 469 ± 172% from baseline in trials 1 and 2, respectively, but was not significantly different between trials (P = 0.46). MAP responses were not significantly different during the two trials (Δ33 ± 3 and Δ38 ± 2 mmHg for trials 1 and 2, respectively). Heart rate responses to IHG were also similar for the two trials (Δ19 ± 2 and Δ22 ± 2 beats/min for trial 1 and trial 2, respectively; Fig. 2). Ratings of perceived effort at fatigue were 19 for both trials.

The placebo group also displayed increases in MSNA, MAP, and heart rate during IHG (Fig. 3). There were no significant alterations in these variables between trials. At fatigue, the rating of perceived effort for both trials was 20.

DHG. There was no significant difference in MSNA responses to DHG observed between trials 1 and 2 of the ketoprofen group (Fig. 2). MSNA increased by 12 ± 2 and 13 ± 3 bursts/30 s from baseline during trials 1 and 2, respectively. Total MSNA increased 332 ± 57 and 427 ± 214% in trials 1 and 2, respectively (P = 0.92). MAP increased similarly for the two trials (Δ31 ± 3 and Δ27 ± 2 mmHg for trials 1 and 2, respectively). Increases in heart rate during DHG were not different between trials (Δ24 ± 2 and Δ25 ± 2 beats/min for trials 1 and 2, respectively; Fig. 2). Ratings of perceived effort recorded during exercise were not significantly different between trials at fatigue (20 and 19 for trials 1 and 2, respectively).

Results in the placebo group for changes in MAP and heart rate during DHG reflected those of the drug group with no statistical significance noted (Fig. 3). There was also no significant change in MSNA responses when expressed in burst frequency; however, total activity was higher during trial 1 than trial 2 (Fig. 3). However, because TxB2 was greater during trial 2, this finding would not support the concept that prostaglandins mediated this response. Ratings of perceived effort for trials 1 and 2 in the placebo group were both 19.

PEMI. Physiological responses to PEMI after IHG and DHG in the ketoprofen and placebo groups are presented in Fig. 4. There were no significant differ-

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**Table 1. Preexercise values for the two experimental groups**

<table>
<thead>
<tr>
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<th>Trial 1</th>
<th></th>
<th>Trial 2</th>
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<tr>
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<td>MAP, mmHg</td>
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Values are means ± SE. IHG, isometric handgrip; DHG, dynamic handgrip; HR, heart rate; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity. *P < 0.05 vs. trial 1.
ences in PEMI responses for any variable when trials 1 and 2 were compared. MSNA and MAP remained significantly elevated above baseline during PEMI for all exercise trials. However, heart rate was elevated above baseline during PEMI after DHG but not after IHG.

**MSNA and MAP relationship to TxB2.** Figure 5 presents the relationship between TxB2 levels and MSNA responses during DHG and IHG. During either exercise mode, there was no relationship between MSNA responses to exercise and TxB2 (r² = 0.13 and 0.05 for dynamic and isometric exercise, respectively). Likewise, there was no relationship between MAP responses to exercise and TxB2 (r² = 0.03 for both dynamic and isometric exercise; Fig. 6). The lack of relationship between either MSNA or MAP with TxB2 persisted in examination of the difference in MSNA and MAP responses during the two exercise trials with changes in TxB2 that were mediated by the drug or placebo and exercise (MSNA, r² = 0.003 and 0.007 for dynamic and isometric exercise, respectively; and MAP, r² = 0.38 and 0.07 for dynamic and isometric exercise, respectively).

**DISCUSSION**

The major new findings of this study are 1) MSNA responses to exercise are not altered by physiological changes in prostaglandin levels; 2) PEMI reflex responses are not changed by different levels of prostaglandins; and 3) no relationship exists between either exercise MSNA or MAP responses and TxB2 levels. Thus the concept that prostaglandins mediate changes in MSNA and MAP during exercise in humans was not validated in our study.

In this study, we sought to determine whether physiological changes in prostaglandin production would affect MSNA responses to exercise and PEMI. Previous studies in humans that have specifically examined the role of prostaglandins on neural reflex responses have been limited only to MAP responses to exercise during
reductions in prostaglandin production. These human studies have produced equivocal results (3, 4). Despite significant alteration of prostaglandin production by ketoprofen administration and exercise, we did not observe any effect on MAP responses to exercise and PEMI. In addition, alterations in prostaglandin production did not elicit changes in exercise-induced increases in MSNA. We were unable to find any relationship between MSNA and MAP responses to exercise and PEMI with TxB2 levels ranging from 13 to 100 pg/ml (Figs. 5 and 6). Thus these findings indicate that physiological changes in prostaglandin production do not alter reflex changes in MSNA and arterial pressure during exercise in humans under the conditions in which we tested the subjects.

Our experimental paradigm provides support for our conclusion that prostaglandins do not mediate reflex changes in MSNA and MAP during exercise. First, ketoprofen significantly reduced plasma TxB2 between exercise trials 1 and 2 without modifying MSNA and MAP responses to fatiguing DHG and IHG. Second, in the placebo group, plasma TxB2 was significantly greater during the second exercise bouts but without changes in the MSNA and MAP responses to exercise. This finding of an elevated TxB2 and thus prostaglandin production during exercise is in agreement with cat studies that have demonstrated increased arachidonic acid levels after isometric contractions (20). Despite this significant increase in prostaglandin production, there were still no changes in MSNA and arterial pressure responses to exercise. It would have been expected that the MSNA responses would be augmented if muscle afferents were sensitive to changes in prostaglandins. Finally, responses during PEMI were not different when TxB2 was altered during the different ketoprofen and placebo exercise trials.

To specifically test the effect of prostaglandins on chemically sensitive muscle afferents, PEMI was performed after both dynamic and isometric exercise. MSNA responses were not different during PEMI in either the ketoprofen or the placebo group regardless of the exercise mode. These findings suggest that chemically sensitive muscle afferents were not affected by changes in prostaglandins produced in this study. This finding is in contrast with animal studies that have demonstrated attenuated group IV activity during isometric contractions after cyclooxygenase inhibition (18, 19). Species and methodological differences (e.g., conscious vs. anesthetized preparation) may explain the divergent results. Unlike the animal studies, we measured sympathetic efferent nerve activity and not afferent nerve activity. Additionally, these animal studies did not report cardiovascular responses with their experimental interventions.

Unlike previous human studies that have used only isometric exercise, we also measured responses to dynamic exercise. It has been shown in the cat that indomethacin attenuates group III muscle afferent activity during static contraction (19, 21). Because group III muscle afferents are predominately thought to be engaged by mechanical distortion, more so than group IV muscle afferents, we believed that, unlike isometric contractions, dynamic contractions would continuously engage mechanically sensitive muscle afferents and give us a better opportunity to examine the impact of prostaglandins on group III muscle afferents in humans. Because MSNA was unchanged during dynamic exercise, this suggests that changes in prostaglandin production do not affect mechanically sensitive muscle afferents in humans.

It has been demonstrated that prostaglandins can inhibit norepinephrine release at the synaptic nerve terminal (5, 6). However, the lack of change in MAP with similar MSNA responses in both experimental groups during either increases or decreases in TxB2 suggests that this did not occur in our study. Other human studies have shown that circulating norepinephrine is comparable during isometric and dynamic exercise after indomethacin administration (3, 22). Thus it appears that prostaglandins do not inhibit norepinephrine release during exercise in humans.

It could be argued that, despite differences in TxB2 observed at the start of trial 1 and 2 in the ketoprofen group, differences did not persist during exercise. In three different subjects, TxB2 was determined before the start and after DHG of trials 1 and 2 with ketoprofen. During trial 1 without ketoprofen, TxB2 increased from 30 ± 13 to 50 ± 12 pg/ml after exercise, whereas, during trial 2 after oral ketoprofen administration, TxB2 was reduced to 16 ± 1 pg/ml before the start of exercise and remained suppressed after exercise (15 ± 1 pg/ml). These data indicate that ketoprofen effectively prevented prostaglandin production during the exercise bouts.

The present study does not preclude the possibility that prostaglandins do contribute to neural reflexes originating from the exercising muscle. Because we did not block prostaglandin production completely, we could not test this possibility. The data from Fontana et al. (4) suggest that some undetermined level of prostaglandins is needed to elicit the full expression of reflex changes during exercise. Our data suggest that this level must be very low. Conversely, our data do not preclude that very high levels of prostaglandins may alter neural reflex responses. But, more importantly,
the present study clearly indicates that physiological increases in prostaglandins from resting levels elicited by exercise do not modify neural reflex responses. In addition, prostaglandins may sensitize muscle afferents to be more responsive to other metabolites (21). Again, our experimental design cannot definitely test this concept because we did not completely block prostaglandin synthesis. However, if this concept is valid, our data suggest that altering levels of prostaglandins across a large physiological range does not change the sensitization of the muscle afferents. Finally, it is possible that, during exercise, central neural mechanisms (i.e., central command) may have modulated or offset any change in muscle afferent feedback, thus obscuring our ability to detect an effect of prostaglandins on MSNA and MAP.

In conclusion, physiological changes in prostaglandin production do not alter sympathetic and cardiovascular responses to forearm exercise in humans. These findings suggest that contraction-induced metabolites other than prostaglandins mediate MSNA responses to exercise in humans.

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