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Local Prostaglandin Blockade Attenuates Muscle Mechanoreflex Mediated Renal Vasoconstriction during Muscle Stretch in Humans

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Abstract

During exercise muscle mechanoreflex mediated sympathoexcitation evokes renal vasoconstriction. Animal studies suggest that prostaglandins generated within the contracting muscle sensitize muscle mechanoreflexes. Thus, we hypothesized that local prostaglandin blockade would attenuate renal vasoconstriction during ischemic muscle stretch. Eleven healthy subjects performed static handgrip before and after local prostaglandin blockade (6 mg ketorolac tromethamine infused into the exercising forearm) via Bier block. Renal blood flow velocity (RBV; Duplex Ultrasound), mean arterial pressure (MAP; Finapres) and heart rate (HR; ECG) were obtained during handgrip, post-handgrip muscle ischemia (PHGMI) followed by PHGMI with passive forearm muscle stretch (PHGMI+stretch). Renal vascular resistance (RVR, calculated as MAP/RBV) was increased from baseline during all paradigms except during PHGMI+stretch after the ketorolac Bier block trial where RVR did not change from baseline. Before Bier block, RVR rose more during PHGMI+stretch than during PHGMI alone ($P < .01$). Similar results were found after a saline Bier block trial ($\Delta 53 \pm 13\%$ vs. $\Delta 35 \pm 10\%$; $P < .01$). However, after ketorolac Bier block, RVR was not greater during PHGMI+stretch than during PHGMI alone ($\Delta 39 \pm 8\%$ vs. $\Delta 40 \pm 12\%$; $P = \text{NS}$). HR and MAP responses were similar during PHGMI and PHGMI+stretch ($P = \text{NS}$). Passive muscle stretch during ischemia augments renal vasoconstriction suggesting that ischemia sensitizes mechanically sensitive afferents. Inhibition of prostaglandin synthesis eliminates this mechanoreceptor sensitization mediated constrictor responses. Thus, mechanoreceptor sensitization in humans is linked to the production of prostaglandins.

Keywords

kidney; exercise; vasoconstriction; prostaglandin; nervous system; sympathetic

Introduction

Exercise activates the sympathetic nervous system (SNS) which contributes to increases in heart rate (HR), blood pressure, ventilation and peripheral vascular resistance. These adjustments are essential for adequate perfusion of vital organs e.g. skeletal muscle, heart and brain (31). Work by Alam and Smirk more than half a century ago “suggested” that during

exercise, muscle metabolites accumulate in active skeletal muscle and when these metabolites are trapped in the muscle during a period of post-exercise circulatory arrest, a pressor reflex is evoked (1), presumably by direct stimulation of metabolite sensitive afferents in the muscle. This response has been termed the “muscle metaboreflex” (3,23,32,38,43). It has been further demonstrated that stimulation of mechanically sensitive afferents during muscle contraction activates the SNS (18,19,52) and evokes vasoconstriction. This response has been termed the “muscle mechanoreflex”. Over the last decade, investigators have consistently demonstrated that contraction induced accumulation of metabolic byproducts including prostaglandins, lactate, ATP, etc. can sensitize mechanosensitive muscle afferent nerves during muscle contraction (22,27,40,45). This process of sensitization can decrease the threshold and increase the frequency of afferent discharge for a given level of mechanical deformation.

During exercise, vasoconstriction occurs in the kidney. At rest, ~one-fourth of the cardiac output flows to the kidney (55). It is known that sympathetically mediated renal vasoconstriction during exercise helps maintain blood pressure as well as helps to redistribute blood flow away from this “inactive” vascular bed towards the “active” contracting muscle (4,8). Reports in anesthetized cats demonstrated that muscle mechanoreflexes play a crucial role in evoking renal sympathoexcitation during muscle contraction (52).

Recently, studies performed in humans suggest that muscle mechanoreflexes are engaged with handgrip and evoke renal vasoconstriction presumably by increasing renal sympathetic tone (30,34). It is not known whether sensitization of muscle mechanoreceptors by metabolic byproducts contribute to the renal vasoconstriction seen in humans. We believe this is an important issue since recent studies suggest that muscle mechanoreflex sensitization may be exaggerated in congestive heart failure and this may contribute to the heightened SNS activation and renal vasoconstriction seen in this disease (28,29,33,46).

A number of prior reports have suggested that prostaglandins play an important role in sensitizing mechanically sensitive muscle afferents (6,16,27,40). In this report we tested the hypothesis that muscle ischemia would sensitize mechanically sensitive afferents. We further suspected that mechanoreceptor sensitization would augment renal vascular resistance (RVR) seen during post-handgrip muscle ischemia (PHGMI). Finally, since prostaglandins are thought to play a role in mechanosensitization of afferents, we hypothesized that prostaglandin inhibition would attenuate the mechanoreceptor sensitization seen with ischemia. We examined renal vasoconstrictor responses during PHGMI alone and during passive forearm muscle stretch with PHGMI in 11 healthy volunteers. Passive muscle stretch engages mechanosensitive muscle afferent nerves (48) without evoking metaboreflex and central command (12,14). Central command is another important neural mechanism resulting in the stimulation of the sympathetic nervous system. It emanates from higher brain center during voluntary exercise and activates autonomic pathways in parallel to the activation of motor neurons (12). To help ensure that prostaglandin inhibition was isolated to the forearm, the protocol was performed before and after ketorolac, a commonly used non-steroidal anti-inflammatory drug that inhibits prostaglandin synthesis, was instilled into the exsanguinated and ischemic forearm via the Bier block method. Our data reveal that passive stretch during ischemia augments renal vasoconstriction. This effect of muscle stretch was eliminated by ketorolac suggesting that muscle mechanoreflexes were sensitized by the local prostaglandins generated during exercise.

Methods

Study Population

Eleven healthy young volunteers (8 Male, 3 female; age 25 ± 1 years, mean body mass index 23 ± 1 Kg/m²) were studied. Each subject signed an informed written consent and a physical

examination was done prior to conducting the study protocols. The protocols were approved by the Institutional Review Board at the Hershey Medical Center. All volunteers were normotensive, nonsmokers and on no medication.

Four among 11 subjects tested were also part of another experiment in which muscle sympathetic nerve activity (MSNA) responses to muscle stretch were examined (Cut et al. companion manuscript).

Measurements

Renal Blood Flow Velocity (RBV)—All subjects were studied in the post absorptive state. Duplex ultrasound (HDI 5000, ATL Ultrasound, Bothell, WA, USA) was used to determine renal blood flow dynamics. The renal artery was scanned using the anterior abdominal approach while the subject was lying supine. A curved-array transducer (2-5 MHz) with a 2.5 MHz pulsed Doppler frequency was used. The probe insonation angle to the renal artery was $<60^\circ$. The focal zone was set at the depth of the renal artery. In order to obtain optimum velocity tracings, the transducer was held in a constant position. Therefore, the data were obtained in the same phase of the respiratory cycle of the respective subject. Care was taken to ensure that the subject did not perform Valsalva maneuvers during the protocols. Each cardiac cycle Doppler tracing was analyzed using the ATL machine's own software (HDI 5000) to obtain mean RBV. Velocity measurements were expressed as cm/sec. It should be noted that blood flow (BF) is a function of mean blood flow velocity (MBV) and vessel cross sectional area ($BF = MBV * \pi r^2$, where r is the vessel radius). Therefore, accurate measurement of vessel diameter is required to measure blood flow. Due to limited spatial resolution of the technique, accurate measurements of renal artery diameter are difficult to obtain in humans. However, using the renal angiography technique, Marraccini et al. reported that large reduction changes in flow velocity evoked by pharmacologic constrictors did not change renal artery diameter (24). We therefore consider RBV to be a valuable index of renal blood flow and this RBV is used in this report as our index of renal blood flow. Beat-by-beat recording of changes in RBV were obtained during the different paradigms. An index of RVR was calculated by dividing mean arterial pressure (MAP) by the corresponding RBV. RVR is expressed in arbitrary units.

Blood Pressure and Heart Rate—Continuous recordings of heart rate (HR; electrocardiogram) and blood pressure (Finapres; Ohmeda, Madison, WI) were obtained during each paradigm. Resting MAP's obtained from the Finapres were verified by an automated sphygmomanometer (Dinamap, Critikon, Tampa, FL). Respiratory excursions were monitored with pneumography.

Force of Muscle Contraction—A force transducer was used to measure the strength of muscle contraction. Non-dominant forearm maximum voluntary handgrip efforts were obtained in triplicate and the highest values were termed the maximal voluntary contraction (MVC).

Thromboxane B2—Venous samples were collected from the antecubital vein of the exercising arm to measure plasma thromboxane B2, a plasma biomarker of prostaglandin synthesis (47). Thromboxane B2 was used to document the effectiveness of blockade of cyclooxygenase (a catalytic enzyme that converts arachidonic acid to prostaglandin). Cyclooxygenase blockade was accomplished by intravenous infusion of ketorolac, a non-selective cyclooxygenase inhibitor (described below). Thromboxane B2 levels were quantified by an enzyme immunoassay (Amersham Biosciences).

Static Exercise Protocol: Fatiguing Static Handgrip Followed by PHGMI and PHGMI+Passive Muscle Stretch—This protocol was designed to determine the

contribution of different neural mechanisms to renal vasoconstriction seen during muscle contraction.

Studies were performed on two separate days. On Day 1, subjects performed the static exercise protocol before (Trial A) and after the ketorolac Bier block procedure (Trial B or ketorolac Bier block trial). To separate the effects of ketorolac from the Bier block procedure itself and to minimize the influence of trial order on the hemodynamic responses, a control study was performed in all subjects on Day 2. On this day, normal saline as opposed to ketorolac was infused during the Bier block. Subjects repeated the same static exercise protocol before (Trial A) and after the saline Bier block procedure (Trial B). Trial B on Day 2 (saline Bier block trial) was considered as a control study of Trial B on Day 1 (i.e. the ketorolac Bier block trial).

All subjects were studied while lying supine on a bed. An intravenous catheter was inserted in the antecubital fossa of the non-dominant arm. Venous blood samples were obtained to measure Thromboxane B2 during baseline and during the PHGMI period in each of the four trials.

Day 1 Studies

Trial A—After instrumentation, 5 min of resting HR, MAP and RBV data were collected. A baseline blood sample was obtained. Each subject then performed static handgrip at 30% of the respective subject's MVC and continued until fatigue. Each subject received visual feedback of the amount of generated tension during static handgrip. At the end of exercise, all subjects graded their perceived level of exertion as 20 (maximum effort) on the Borg scale (2). Immediately prior to stopping exercise, PHGMI was initiated by inflating a previously placed blood pressure cuff around the arm to ~250 mmHg. The cuff was kept inflated for 4 min. PHGMI traps the metabolites that are generated within the exercising arm, and activates the muscle metaboreflex (3,23,43). Approximately 2½ min after initiating PHGMI, one of the investigators applied passive forearm muscle stretch to each subject by extending the wrist joint against resistance using a flexible device that was fitted to the forearm. We could not estimate the actual force of passive stretch applied in all subjects. However, in the last four subjects a device was used allowing us to quantify the level of force generated. Forearm flexion force was measured using an Imada® DPS-220 digital force gauge (Imada Inc., Northbrook IL, USA). This portable device performed all signal transduction and conditioning internally and displayed data on its surface. In the last four subjects studied, the force data from this portable device were also displayed on an LCD panel for direct monitoring by the investigators while outputting an analog signal via a custom cable to PowerLab®. The average force measured in these subjects was $5.28 \pm .11$ Kg (range, 4.25-6.52 Kg). Importantly, care was taken in all subjects to ensure that the subject did not feel any pain during the procedure. Sustained passive stretch was continued for ~1.5 min. Then the arm cuff was deflated and the subject rested for ~15 min.

Bier Block—After 10-15 min of recovery from Trial A, the Bier block procedure was utilized to regionally administer ketorolac tromethamine, a potent nonsteroidal anti-inflammatory drug to block prostaglandin synthesis in the forearm (5). Although a specific mechanism of action of ketorolac (marketed as toradol) is not known, it is thought that ketorolac inhibits prostaglandin synthesis by competitive blockade of the enzyme cyclooxygenase which converts arachidonic acid to prostaglandin. Like many other non-steroidal anti-inflammatory drugs, ketorolac is a non-selective cyclooxygenase inhibitor. It is a parenteral agent that is commonly used for moderate to severe acute pain management situations such as for post surgical analgesia (10,13). It is also commonly used in ophthalmic preparation for seasonal conjunctivitis and inflammation following cataract extraction

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http://www.uptodateonline.com/utd/content/topic.do?topicKey=drug_a_k/138848&selectedTitle=2~150&source=s). The half-life of ketorolac is ~6 hours (http://www.rxlist.com/cgi/generic/ketor_cp.htm).

Beginning at the hand, the arm was elevated and bandaged with a tight elastic wrapping. This was done in order to “drain” the forearm vasculature. The pneumatic cuff on the upper arm was then inflated to 250 mmHg and the bandage was removed. Thereafter, 6 mg ketorolac tromethamine (in 40 ml of saline) was infused into the ischemic arm via a venous catheter. The cuff on the upper arm remained inflated for 20 min to allow the ketorolac to distribute itself within the previously emptied vascular bed and then to diffuse into the forearm tissue. The cuff was deflated and the subject rested for 15-20 min before performing *Trial B*.

Trial B (Ketorolac Bier Block Trial)—Another 5 min of baseline data were collected. During this trial, subjects repeated the same static exercise protocol as in Trial A.

Day 2 Studies

Trial A—Trial A on Day 2 was identical to Trial A performed on Day 1.

During the Bier block procedure (described above), 40 ml saline (no ketorolac) was infused into the arm.

Trial B (saline Bier block trial)—Trial B on Day 2 was identical to Trial B on Day 1.

Data Analysis and Statistics

Beat-by-beat sequential analysis of HR, MAP, RBV and RVR were performed for all subjects. Baseline values for each parameter were considered as the average data obtained during the 5-min rest period preceding the handgrip protocol in each trial. Variables during fatiguing handgrip were considered as the average values obtained during the last 30 s period before the subject fatigued. Variables during PHGMI and passive stretch with PHGMI were considered as the average values obtained across the time period of each intervention.

Finapres recordings could not be obtained from one subject during Day 2 studies. Therefore, the Day 2 data are presented for 10 subjects. It also should be noted that in the female subjects, the effects of the different phases of menstrual cycle were not controlled for in these studies.

Data are presented as mean $\pm$ SE. Repeated measure one-way ANOVA followed by a Dunnett's tests were used to determine significance for each protocol. Paired t-tests were used to compare values within the same subject. *P* value <0.05 was considered significant.

Results

Baseline Measurements

No significant differences were found in the baseline variables before and after ketorolac or before and after saline Bier block trials (*P* = NS). However, MAP after the ketorolac infusion was significantly higher than the baseline MAP before ketorolac (85 ± 3 vs. 81 ± 4 mmHg *P* = .044).

Prostaglandin Inhibition

Table 1 shows values of plasma Thromboxane B2 during the studies on Day 1 and Day 2. At rest, plasma Thromboxane B2 level was reduced (~75% of baseline values) after the ketorolac infusion. After exercise, Thromboxane B2 level was increased from the corresponding baseline values during Trials A on both Day 1 and Day 2 whereas no increments in Thromboxane B2 were found during the ketorolac Bier block trial on Day 1. On Day 2 during Trial B, plasma

Thromboxane B2 level was not significantly higher than baseline although a trend was present ($P = .08$).

The half-life of ketorolac tromethamine is ~6 hr (http://www.rxlist.com/cgi/generic/ketor_cp.htm). In 6 out of 11 subjects, Day 2 trials were performed about one month after the ketorolac trial on Day 1. Four subjects performed Day 2 trials more than a month after the ketorolac trial. In one subject, the second study was performed 18 days after the first. By the time this latter subject performed studies on Day 2, the drug had gone through 72 half-lives (4 half life's in one day *18).

Fatiguing Static Handgrip

Significant increases in RVR, MAP and HR were noted during fatiguing handgrip in all four trials (Table 2). Significant decreases in RBV were also noted during fatiguing handgrip (Table 2).

Increases in RVR, MAP and HR at the time of fatigue were not different before and after ketorolac infusion ($P = \text{NS}$; data not shown).

PHGMI With and Without Passive Muscle Stretch

RVR was increased from baseline during both PHGMI and PHGMI+muscle stretch in all trials except during the ketorolac Bier block trial where RVR during PHGMI+muscle stretch was not increased as compared to baseline (Table 2). Furthermore, increases in RVR during PHGMI+muscle stretch were greater than the RVR responses during PHGMI alone in all trials ($P < .03$) except during the ketorolac Bier block trial, where RVR responses to PHGMI+muscle stretch were not different than the responses to PHGMI alone ($P = \text{NS}$; Figure 1). Significant paradigm effects were noted for RBV and MAP in all four trials ($P < .001$; Table 2). HR did not change with PHGMI or during PHGMI+muscle stretch in any of the four trials (Table 2).

Discussion

Study Findings

The important new finding in the present report is that local prostaglandin blockade attenuated muscle mechanoreflex mediated renal vasoconstriction during muscle stretch in humans. Our findings are consistent with the concept that prostaglandins can sensitize mechanosensitive muscle afferent nerves during exercise. The remainder of this discussion will focus on the background, the study design and the clinical implications of our findings.

Background and Study Design

Studies in anesthetized cats have shown that thinly myelinated group III and group IV muscle afferent nerves comprise the afferent arm of the muscle reflex that is evoked during muscle contraction (26). Activation of group III afferents occurs mainly due to mechanical deformation of the receptive fields whereas group IV afferents are activated by metabolic byproducts of muscle contraction (19). One important group of metabolites noted in several prior animal reports are the prostaglandins (17,39,49,51). These reports suggest that cyclooxygenase products of arachidonic acid metabolism (e.g. prostaglandin) can sensitize group III afferents during muscle contraction (16,40), and hence muscle mechanoreflexes may be sensitized by prostaglandins. Reports in humans, however, are equivocal. Studies performed in our laboratory and in others observed decreased MSNA (6) and decreased pressor responses (11) to static handgrip protocol after prostaglandin inhibition, while studies performed by other groups observed no influence of prostaglandin inhibition on MSNA or cardiovascular variable during static handgrip (7,9). Ragan et al. performed studies in decerebrate cats and observed diminished pressor responses with muscle contraction after topical application of a salicylate

based analgesic balm (inhibits prostaglandin synthesis). The authors suggested that attenuated muscle reflexes were responsible for the observed diminished pressor response (37). A recent report in healthy humans measured MSNA responses during low-level rhythmic handgrip in an effort to activate primarily muscle mechanoreflexes. These investigators noted that muscle mechanoreflex mediated increases in MSNA were markedly diminished after prostaglandin inhibition suggesting that muscle mechanoreceptors were sensitized by prostaglandins (27). On the basis of the observations made in these prior works, we designed a study to examine renal vasoconstrictor responses during fatiguing static handgrip, PHGMI and passive stretch +PHGMI before and after prostaglandin inhibition by ketorolac. The paradigm of passive stretch was designed with the speculation that muscle mechanoreflexes will be engaged during muscle stretch since the animal reports suggested that muscle stretch activates primarily mechanosensitive group III afferent nerves (48). Moreover, studies in anesthetized cats (25) as well as in decerebrate rats (20) examined renal vascular responses during muscle stretch and observed renal vasoconstriction that was mediated by muscle reflexes. Additionally, studies in decerebrate cats suggest that muscle reflex mediated renal sympathetic nerve activation was accentuated when the contracting skeletal muscle was rendered ischemic by limb circulatory arrest (15). Therefore, we speculated that in humans activation of the mechanoreceptors during passive stretch would be enhanced by the metabolic products during PHGMI and would lead to enhanced renal vasoconstriction.

In the present report, we noted significant increases in Thromboxane B2 after handgrip exercise during the control trials suggesting that handgrip exercise led to increased local prostaglandin synthesis within the exercising arm (54). As expected, Thromboxane B2 values were decreased after the ketorolac infusion during baseline as well as after exercise suggesting that prostaglandin synthesis was attenuated after ketorolac administration (Table 1). These observations are consistent with previous work (5,21,35,54).

The data in the present report show that passive muscle stretch during PHGMI led to increased RVR in all trials except the ketorolac Bier block trial. These data suggest that muscle mechanoreflexes were sensitized by prostaglandins. It should be mentioned that work by Cui et al. described in the companion manuscript examined muscle sympathetic nerve activity responses to muscle stretch. This report suggests that muscle mechanoreflexes were sensitized by prostaglandins and augmented the MSNA response.

However, RVR responses at the time of fatigue were not different before or after ketorolac infusion. A recent report from our lab observed attenuated efferent sympathetic drive to the muscle during handgrip after ketorolac infusion (6). The exact reason why our data did not show attenuated renal vasoconstrictor responses during handgrip after ketorolac infusion is not clear. However, it should be noted that during fatiguing handgrip, mechanisms aside from muscle mechanoreflexes contribute to the renal vasoconstriction (34). These other mechanisms include central command and muscle metaboreflexes. Importantly, studies performed in decerebrate cats showed that either central command or muscle reflex mechanisms could induce efferent sympathetic drive to the kidney (15). Therefore, we speculate that after ketorolac, other neural mechanisms compensated for reduced mechanoreceptor engagement.

Clinical Implications

In the present report our primary objective was to evaluate the renal vascular responses evoked when muscle mechanoreflexes were sensitized by prostaglandins generated by muscle contraction. To this end we selectively engaged mechanoreflexes with muscle stretch performed during post handgrip muscle ischemia. It should be noted that renal vasoconstriction is exaggerated during exercise in heart failure patients (55). Moreover, recent data suggest that muscle mechanoreflexes play a crucial role in evoking exaggerated renal vasoconstriction during exercise in heart failure (29,33). Wilson et al. demonstrated that muscle metabolism

during exercise is abnormal in heart failure patients (53). Additionally, studies performed in heart failure patients observed a direct correlation between increased exercise induced intramuscular concentrations of prostaglandins and exaggerated ventilatory responses to exercise (42). Therefore, based on these prior observations and the data in the present report, we postulate that a link exists between prostaglandin generation within the contracting muscle and exaggerated muscle mechanoreflex mediated renal vasoconstriction in heart failure patients. It should be noted that the increased renal vasoconstriction that we observed during passive stretch involved a relatively small muscle mass. It is not known whether the magnitude of the renal responses will be greater when there is a larger muscle mass involved. However, it is plausible that the magnitude of renal vasoconstriction in heart failure patients would be greatly enhanced when a larger group of muscle is engaged in exercise since more nerve afferents would be engaged and the muscle mechanoreceptors may be sensitized (22,27,40, 45). Future studies in heart failure patients will be necessary to establish these postulates. Moreover, performing similar experiments in a heart failure patient population in conjunction with exercise training will also shed light on the pathophysiology of this disease. Previous reports have indicated that exercise training reduces muscle mechanoreflexes in healthy humans (44) as well as reduces sympathetic activity (41) and improves muscle metabolism (50) in heart failure patients. Therefore, it is conceivable that exercise training in these patients would attenuate the exaggerated muscle mechanoreceptor activation/sensitization. Consequently, these would reduce renal vasoconstriction in heart failure. We believe this issue is of major clinical and functional significance since exaggerated renal vasoconstriction is known to be responsible for enhanced retention of sodium and water by the kidney and leads to further deterioration of the clinical sign/symptoms in the heart failure patients (36,55).

In conclusion, data from the present report support our hypothesis and suggest that locally generated prostaglandins within the exercising muscle enhance muscle mechanoreflex mediated renal vasoconstriction in humans.

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Figure 1.

Data are presented as mean $\pm$ SE. Data are shown as % change from baseline in renal vascular resistance index (RVR; Y-axis) during post-handgrip muscle ischemia (PHGMI) and post-handgrip muscle ischemia with stretch (PHGMI+stretch) before (**Panel A**) and after ketorolac (**Panel B**) ($n = 11$) and before (**Panel C**) and after saline (**Panel D**) ($n = 10$) Bier block procedure. (*) indicates significant differences (P value $< .05$) between the RVR responses during PHGMI and PHGMI+stretch.

Table 1
Thromboxane B₂ (pg/ml) Data at Baseline and Post-Handgrip

	Baseline (pg/ml)	Post-Handgrip (pg/ml)
Before Ketorolac	183.0 ± 45.1	344.1 ± 72.5 [*]
After Ketorolac	39.7 ± 4.4 [†]	31.3 ± 3.0 [†]
Before Saline	178.4 ± 40.4	287.8 ± 60.2 [*]
After Saline	167.3 ± 33.2	210.4 ± 28.9

Data are presented as mean ± SE.

^{*} ($P < 0.04$) significantly different from respective baseline values.

[†] ($P < 0.02$) significantly different than the corresponding pre ketorolac values.

Table 2

Data from Static Handgrip Protocol

	Base	FHG	Before Ketorolac PHGMI	PHGMI +Stretch	P <	Base	FHG	After Ketorolac PHGMI	PHGMI +Stretch	P <
HR	56 ± 2	79 ± 2 *	61 ± 2	61 ± 2	0.001	56 ± 1	79 ± 3 *	59 ± 2	59 ± 2	0.001
MAP	81 ± 4	107 ± 5 *	102 ± 6 *	102 ± 5 *	0.001	85 ± 3	111 ± 7 *	103 ± 6 *	103 ± 5 *	0.001
RBV	53.7 ± 2.3	46.2 ± 2.6 *	50.9 ± 2.3	47.9 ± 2.1 *	0.001	54.6 ± 2.4	43.6 ± 2.3 *	48.6 ± 2.2	48.2 ± 2.0	0.001
RVR	1.53 ± .09	2.41 ± .21 *	2.05 ± .16 *	2.18 ± .17 *	0.001	1.58 ± 0.10	2.67 ± .28 *	2.19 ± .20 *	2.18 ± .16	0.001
			Before Saline PHGMI	PHGMI +Stretch	P <	Base	FHG	After Saline PHGMI	PHGMI +Stretch	P <
HR	57 ± 2	79 ± 4 *	61 ± 3	61 ± 2	0.001	58 ± 2	77 ± 3 *	60 ± 2	61 ± 2	0.001
MAP	82 ± 3	106 ± 5 *	103 ± 4 *	104 ± 4 *	0.001	81 ± 3	109 ± 5 *	104 ± 5 *	106 ± 6 *	0.001
RBV	49.2 ± 2.7	41.8 ± 2.4 *	46.6 ± 3.2	42.9 ± 2.8	0.001	48.0 ± 2.8	39.8 ± 3.1 *	47.4 ± 3.8	42.3 ± 3.0	0.001
RVR	1.71 ± .11	2.67 ± .20 *	2.30 ± .18 *	2.53 ± .23 *	0.001	1.74 ± .12	2.95 ± .31 *	2.36 ± .25 *	2.70 ± .32 *	0.002

All hemodynamics data [HR, heart rate (beats/min); MAP, mean arterial pressure (mmHg); RBV, renal blood flow velocity (cm/sec); and RVR, renal vascular resistance index] are presented as mean ± SE. Data are shown during baseline (base), fatiguing handgrip (FHG), post-handgrip muscle ischemia (PHGMI) and post-handgrip muscle ischemia with stretch (PHGMI+stretch). P value on the right column indicates the paradigm effect from a one-way analysis of variance.

* denotes different from baseline using the Dunnett's test.