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Contributions of acetylcholine and nitric oxide to forearm blood flow at exercise onset and recovery

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Shoemaker, J. K., J. R. Halliwill, R. L. Hughson, and M. J. Joyner. Contributions of acetylcholine and nitric oxide to forearm blood flow at exercise onset and recovery. *Am. J. Physiol.* 273 (*Heart Circ. Physiol.* 42): H2388–H2395, 1997.— The contributions of acetylcholine and/or nitric oxide (NO) to the rapid changes in human forearm blood flow (FBF) at the onset and recovery from mild exercise were studied in eight subjects. Rhythmic handgrip contractions were performed during brachial artery infusions of saline (2 ml/min; control), atropine (0.2 mg over 3 min), to block acetylcholine binding to muscarinic receptors, or atropine + N^G-monomethyl-L-arginine (L-NMMA; 4 mg/min for 4 min), to additionally inhibit NO synthase. Brachial artery mean blood velocity (MBV; pulsed Doppler ultrasound) and diameter (echo Doppler) were measured continuously, and FBF was calculated. Atropine reduced acetylcholine-induced increases in FBF by ~71% ($P < 0.05$). FBF at rest was reduced by atropine and further reduced with atropine + L-NMMA. Both drug conditions reduced FBF during exercise by ~10% compared with control, with no difference between drug treatments. Brachial artery diameter was unchanged from rest by exercise, recovery, and drug treatments. Neither drug treatment altered the rate or magnitude of the increase in FBF above rest. Peak FBF after exercise was reduced by atropine and atropine + L-NMMA. Total FBF during 5 min of recovery was reduced with atropine + L-NMMA compared with control and atropine. The results suggest that *1*) acetylcholine and NO mechanisms additively contribute to FBF levels at rest, *2*) a cholinergic mechanism adjusts the absolute FBF levels during exercise, *3*) neither acetylcholine nor NO is essential to observe the normal time course or magnitude of the exercise response, and *4*) NO contributes to the FBF response during recovery from exercise.

brachial artery; pulsed Doppler; echo Doppler; vasodilation; atropine; N^G-monomethyl-L-arginine

BLOOD FLOW TO EXERCISING LIMBS increases rapidly to a steady level after the onset of voluntary submaximal exercise. The mechanisms responsible for the increase in blood flow include the action of the muscle pump (27) and a rapid vasodilation (6, 16, 33). The mediators for the early vasodilation are unknown, and it is not clear whether the factors that initiate the dilation are the same as or different from those that maintain flow during steady-state contractions or recovery from exercise (26). Of the various contributors to vascular tone, neither muscle metabolites (4) nor prostaglandins (29) are believed to contribute to the rapid vasodilation with exercise onset. In humans, nitric oxide (NO) is known to affect limb blood flow both at rest (22, 35) and after steady-state exercise (8), suggesting that this compound may also have a role during the transition between rest and exercise.

Anrep and von Saalfeld (1) reported that stimulation of the motor nerve to dog muscle that had been paralyzed with curare did not cause any increase in blood flow. However, on the basis of in vitro observations of hamster muscle preparations, Segal and Kurjiaka (25) have proposed that the release of acetylcholine from the neuromuscular junction may diffuse to neighboring arteriolar or capillary endothelial tissue and initiate a rapidly conducted vasodilatory signal that would coordinate blood flow distribution to the active tissue. Although the anatomic and pharmacological evidence supports a role for this proposition with in vitro hamster muscle preparations (21, 25), there is, as yet, no evidence for a cholinergic mechanism in mediating the rapid changes in flow with voluntary human exercise.

Therefore, the purpose of this study was to investigate the roles of acetylcholine and NO in regulating the time course and magnitude of change in forearm blood flow (FBF) during and after voluntary rhythmic exercise in humans. Neurally released acetylcholine has been shown to stimulate muscarinic receptors on the vascular endothelium leading to the release of NO (5). Therefore, the cholinergic mechanism proposed by Segal and Kurjiaka (25) might act through NO release. We addressed this issue by studying the blood flow responses to rhythmic handgrip exercise before and after locally blocking muscarinic receptors with atropine followed by a combined blockade of muscarinic receptors plus inhibition of NO synthase [atropine + N^G-monomethyl-L-arginine (L-NMMA)].

METHODS

Subjects

Eight healthy subjects (6 male and 2 female) participated in this study after giving written informed consent to procedures that had been approved by the Institutional Human Subjects Committee at the Mayo Clinic (Rochester, MN), where the study was performed. The subjects were 25.5 ± 1.6 yr old and 177 ± 1.7 cm in height and weighed 74.8 ± 3.0 kg (means ± SE).

Subject Instrumentation

Thirty minutes before any exercise, a 20-gauge, 4.45-cm catheter (Arrow International, Reading, PA) was inserted under local anesthesia into the brachial artery of the active arm. A three-port connector was placed in series with the catheter. One port was used for brachial arterial blood pressure measures, and the other two ports were used for drug infusions. Room temperature was held constant at 21°C. The subjects were asked to abstain from food for 3 h and from alcohol and caffeine for 24 h before testing.

Exercise

While supine, the subjects performed dynamic handgrip exercise with the nondominant forearm by lifting and lowering a 4.4-kg weight (~9.5% of maximal voluntary isometric contraction) a distance of 5 cm in a work-to-rest ratio of 1 s/2 s. The weight was lifted in ~0.5 s and lowered over 0.5 s. The experimental trials included an observation period during which signals were monitored to ensure a stable baseline. After this period, data were collected during 1 min of rest followed by a step increase in work rate lasting between 5 and 9 min, followed by 5 min of recovery. The time course of the FBF response between rest and steady-state exercise was determined from the initial 5 min of contractions. The duration of the exercise was extended beyond 5 min so that drugs could be infused during exercise. This was done to ensure that the drugs reached the vessels that were dilated during exercise and also to determine whether either acetylcholine or NO contributed to the ongoing dilation during contractions. The exercising arm was positioned so that forearm muscles were ~10 cm below the level of the heart. Each subject was familiarized with the experimental design, exercise protocol, and data collection procedures during a separate orientation session.

Experimental Sequence

All subjects completed the same test sequence. Three experimental conditions were used, corresponding to control, atropine, and atropine + L-NMMA adminstration. Within each condition, two repeated trials were performed so that two on- and off-transient phases could be ensemble averaged together (Fig. 1). The control condition was performed first with saline infused at 2 ml/min during rest, during exercise performed for 5 min, and during the 5-min recovery period. After the first trial, acetylcholine was infused under resting conditions (32 μg/min) to test the normal hyperemic response to this vasodilator. In the second trial of the control condition, the exercise was continued for 8 min, 0.2 mg of atropine was infused over the last 3 min of steady-state exercise to block muscarinic receptors, and the recovery response was recorded. This initiated the atropine condition. After the second trial, acetycholine was infused again under resting conditions to assess the effectiveness of the atropine treatment. After a supplemental dose of atropine (0.05 mg) given at rest, the third trial was performed to provide the first on- and a second off-transient response under the influence of atropine. Before the fourth trial, a supplemental dose of atropine (0.05 mg) was given at rest. The fourth trial provided the second on-transient phase for atropine treatment but was extended to 9 min of exercise. L-NMMA, to inhibit NO synthase, was infused at 4 mg/min over the final 4 min of exercise and the recovery phase of the fourth trial. This initiated the atropine + L-NMMA condition and provided the first off-transient response under the influence of combined muscarinic receptor and NO synthase blockade. A fifth and a sixth trial, each including 5 min of exercise and 5 min of recovery, were then performed with continuous infusions of L-NMMA (1 mg/min) to account for any drug washout. Also, a small dose of atropine (0.05 mg) was given before each of these last two trials to ensure that muscarinic receptors remained blocked. Details of drug preparation and infusion have been described earlier (7, 8). The drugs were infused during exercise to enhance their distribution to the microvascular units recruited during contractions. Although this sequence of exercise trials and drug interventions was complex, it permitted us to make measures of the rest-to-exercise on-transition at the onset of exercise and the exercise-to-rest off-transition during recovery from the various drug interventions.

Approximately 20 min of recovery rest occurred between each trial. The control, atropine, and atropine + L-NMMA conditions could not be counterbalanced in the current study because of the prolonged effects that both atropine and L-NMMA might have on blood flow (9, 24).

Blood Flow Data Collection and Analysis

FBF was determined from the combined measures of brachial artery diameter and mean blood velocity (MBV). The echo-Doppler (Toshiba Model SSH-140A) image of the brachial artery was collected continuously in real time during the second trial of each experimental condition. A hand-held 7.5-MHz linear probe, operating in B mode, was positioned over the brachial artery ~9 cm proximal to the medial epicondyle. The imaged data were stored on VHS tape for analysis. An estimate of arterial diameter was made from the average of three measurements each at 20-s intervals during 1 min of rest, at 5-s intervals between 0 and 30 s of exercise, at 10-s intervals between 30 and 60 s of exercise, and also at 90, 120, 180, 240, and 300 s of exercise. All diameter measurements were made during diastole in a muscle relaxation phase.

In all trials, brachial artery MBV was determined from the spectra of the pulsed Doppler ultrasound (Multigon, model 500V) signal using a flat 4-MHz probe fixed to the skin over the brachial artery immediately proximal to the catheter. Previous pilot experiments confirmed that catheter placement and saline infusion did not affect the MBV response at rest or during exercise. Beat-by-beat MBV was calculated as the average of the instantaneous MBV values over each cardiac cycle using the QRS complex of the electrocardiogram tracing to signal the end of one blood pulse wave and the beginning of the next. The procedure for processing the Doppler shift spectrum has been described previously (28, 30). The beat-by-beat MBV data from the repeated trials for the on- and off-transient phases of each condition were time aligned and ensemble averaged over 3-s time bins to include a contraction and relaxation phase in each value and thereby reduce contraction-induced variability (31).

The best fits of the MBV data were obtained by a nonlinear least-squares curve fitting procedure using a one- or two-component exponential model as previously described (30). Selection of the one- or two-component model was based solely on best fit criteria. This approach produced the best estimate of the true physiological response after it was recognized that some of the between-sample point variability was probably due to random variation caused by probe movement and muscle contraction. From the modeling parameters, the mean response time for a particular MBV data set was determined. The mean response time is the time required to achieve 63% of the increase in MBV from rest to steady-state exercise levels.

From the modeled data sets, MBV values were obtained at the same times used for the diameter estimations indicated above. Limb blood flow at each time was calculated as FBF = MBV $\times$ πr^2, where r is the vessel radius. The MBV and diameter measures were made proximal to the arterial catheter so that blood flow measures were made upstream from the infusion site.

Statistics

The effect of drug and exercise time on diameter, MBV, and FBF responses was assessed by a repeated-measures two-way analysis of variance (ANOVA; SAS) with planned comparisons for the effect of drug treatment on FBF under resting conditions. For FBF, we reasoned that a statistically signifi-

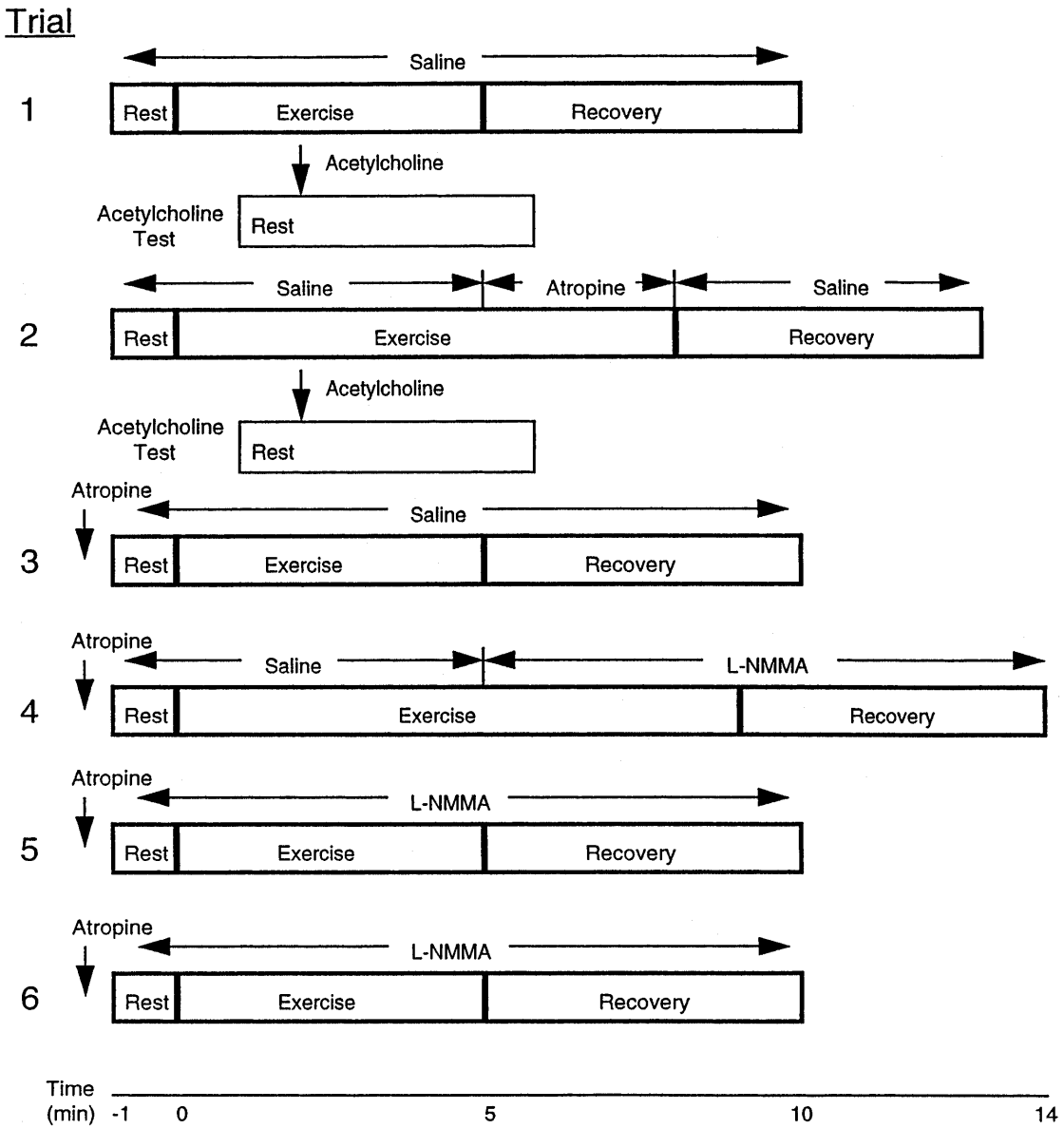

Fig. 1. Experimental design. Subjects performed 2 repeated trials in each of 3 drug treatment conditions to study effects of muscarinic blockade and nitric oxide synthase inhibition on blood flow kinetic responses during on- and off-transitions to submaximal rhythmic handgrip exercise. In *trials 1* and *2*, saline was infused. Contraction phase of *trial 2* was extended by 3 min, during which time saline infusion was stopped and atropine (Atr) was infused to block muscarinic receptors. Contraction phase of *trial 4* was extended by 4 min for treatment with N^G-monomethyl-L-arginine (L-NMMA) for nitric oxide synthase inhibition. L-NMMA infusion continued at a reduced dose during *trials 5* and *6*.

cant drug × time interaction would indicate a difference in on-transient kinetics. Also, the effect of drug treatment on the time course of change in MBV from rest to steady-state exercise was determined by comparing the mean response times for this variable using a one-way ANOVA. The level of statistical significance was set at $P < 0.05$, and any significant findings were further analyzed with Student-Newman-Keuls post hoc test. All data are means ± SE.

RESULTS

Forearm Blood Flow

Rest. Brachial artery diameters at rest were 4.2 ± 0.2 mm in each of the control, atropine, and atropine + L-NMMA trials ($P > 0.05$). MBV at rest was reduced from 6.52 ± 0.6 cm/s in control to 5.19 ± 0.5 cm/s by atropine ($P < 0.05$) and was further reduced by atropine + L-NMMA to 4.72 ± 0.4 cm/s ($P < 0.05$). FBF at rest was reduced from 55.0 ± 6.6 ml/min in control to 46.2 ± 4.4 ml/min by atropine ($P < 0.05$) and was further reduced by atropine + L-NMMA to 38.6 ± 4.5 ml/min ($P < 0.05$; Fig. 2).

Acetylcholine tests: Effect of atropine. The increase in FBF above rest with acetylcholine infusions was reduced by ~71% from 128 ± 20 ml/min under control conditions to 36.9 ± 9.6 ml/min after the atropine treatment ($P < 0.05$). There was no evidence of an effect

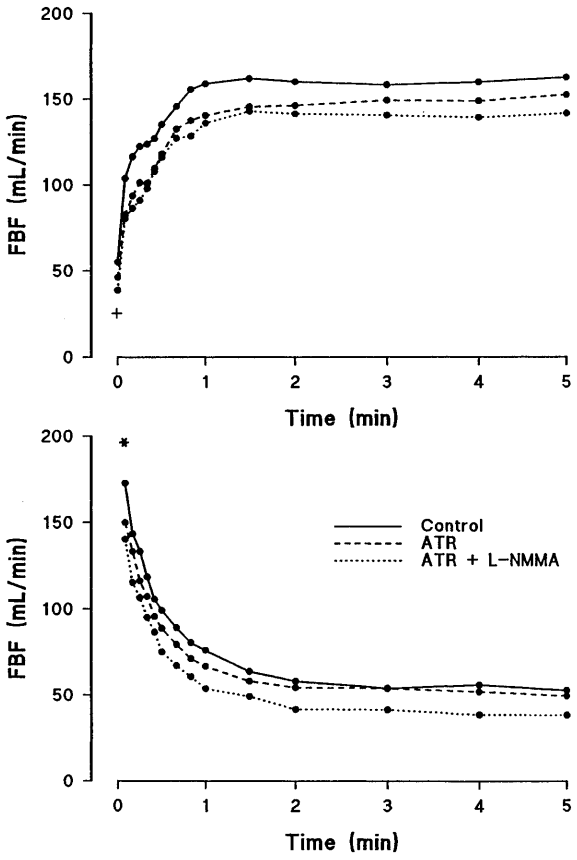

Fig. 2. Forearm blood flow (FBF) response to handgrip exercise was differentially affected by intra-arterial infusions of Atr and Atr + L-NMMA during rest and on-transient (*top*; $n = 8$) and off-transient (*bottom*; $n = 7$) phases. Rest: +, resting FBF was significantly reduced from control by Atr and was further reduced by Atr + L-NMMA ($P < 0.05$). On-transient phase: a main effect of drug treatment was observed during exercise where control FBF was greater than both Atr and Atr + L-NMMA conditions ($P < 0.05$). Atr and Atr + L-NMMA exercise FBF responses were not different ($P > 0.05$). Exercise commenced at *time 0*. SE for on-transient data points ranged from 4.38 to 6.6 at rest and from 9.03 to 15.4 during contractions. Off-transient phase: *, peak recovery flow for control was significantly greater than both Atr and Atr + L-NMMA ($P < 0.05$), which were similar. For overall off-transient flow response, a main effect of drug condition was observed where both control and Atr FBF were greater than Atr + L-NMMA FBF ($P < 0.05$). SE for off-transient data points ranged from 9.96 to 12.18 at 5 min after contractions and from 2.87 to 4.25 late in recovery.

of pharmacological interventions on central hemodynamics, because the resting heart rates before each condition were 62 ± 3, 55 ± 2, and 57 ± 3 beats/min in control, atropine, and atropine + L-NMMA conditions, respectively ($P > 0.05$).

On-transient phase. Diameter measurements during and after exercise were unchanged from rest. Also, drug treatment did not alter diameter measurements at rest or during exercise. The MBV mean response times for control (17.6 ± 2.9 s), atropine (16.9 ± 2.7 s), and atropine + L-NMMA (17.7 ± 2.5 s) were not different.

During exercise, FBF was reduced from control by both atropine and atropine + L-NMMA treatments ($P < 0.05$), but flows during the two drug conditions were not different (Fig. 2). When the increases in FBF above resting levels were compared, atropine and atropine + L-NMMA responses were not different from control. The FBF drug × time interaction was not significant ($P > 0.05$), suggesting that the rate of increase in FBF was not different across experimental conditions.

Individual responses. The consistency of the results across subjects is illustrated by the individual responses of FBF at rest, during the on-transient phase at 10 s after the exercise onset, and at 5 min of exercise with the three drug conditions (Fig. 3). Although a main effect of atropine and atropine + L-NMMA on FBF was found relative to control, it is clear that the effects of atropine and atropine + L-NMMA are most apparent at rest, where atropine reduced FBF in seven of eight subjects and the addition of L-NMMA caused a further reduction in six of eight subjects. At 10 s after the onset of exercise, atropine resulted in an attenuated FBF in seven of eight subjects and the addition of L-NMMA further diminished FBF in only four of eight subjects. In the last minute of exercise, the FBF responses become more variable among subjects, likely because of the contribution of metabolic vasodilatory signals in possible combination with acetylcholine and NO.

Effect of drug treatment on steady-state MBV. The infusion of atropine or L-NMMA during contractions did not alter exercise MBV (Fig. 4).

Off-transient phase. In one subject, the recovery FBF response was highly erratic, perhaps because of difficulty with probe placement; therefore, this subject was not included in the analysis of the off-transient FBF response. For the remaining seven subjects, the first recovery blood flow value was calculated at 5 s after the cessation of exercise. Compared with control (173 ± 10 ml/min), this postexercise "peak" FBF was reduced by both atropine (150 ± 12 ml/min) and atropine + L-NMMA (140 ± 12 ml/min) ($P < 0.05$), but the peak FBF values for the two drug conditions were not different. Overall, a main effect of condition was observed where the FBF during recovery after atropine + L-NMMA was less than both control and atropine conditions ($P < 0.05$; Fig. 2).

The recovery FBF response was also analyzed by integrating the FBF data over the 5-min recovery period to derive the total postexercise flow with a baseline of 0 (Fig. 5). The total postexercise flow with atropine + L-NMMA (243 ± 14 ml) was significantly reduced ($P < 0.05$) from both control (326 ± 22 ml) and atropine (304 ± 23 ml), which were not different.

Brachial Artery Blood Pressure

Mean arterial blood pressure (MAP), measured in the brachial artery of the active arm, was not affected by the different drug treatments at rest or during exercise. For example, the average resting MAP for the control, atropine, and atropine + L-NMMA conditions was 95 ± 2, 94 ± 2 ,and 97 ± 2 mmHg, respectively ($P > 0.05$). Corresponding values for MAP during the last 30 s of exercise were 92 ± 2, 92 ± 2 and 94 ± 2 mmHg ($P > 0.05$). These data suggest that the observed changes in FBF with the drug treatments were not caused by differences in perfusion pressure but by altered forearm vascular tone.

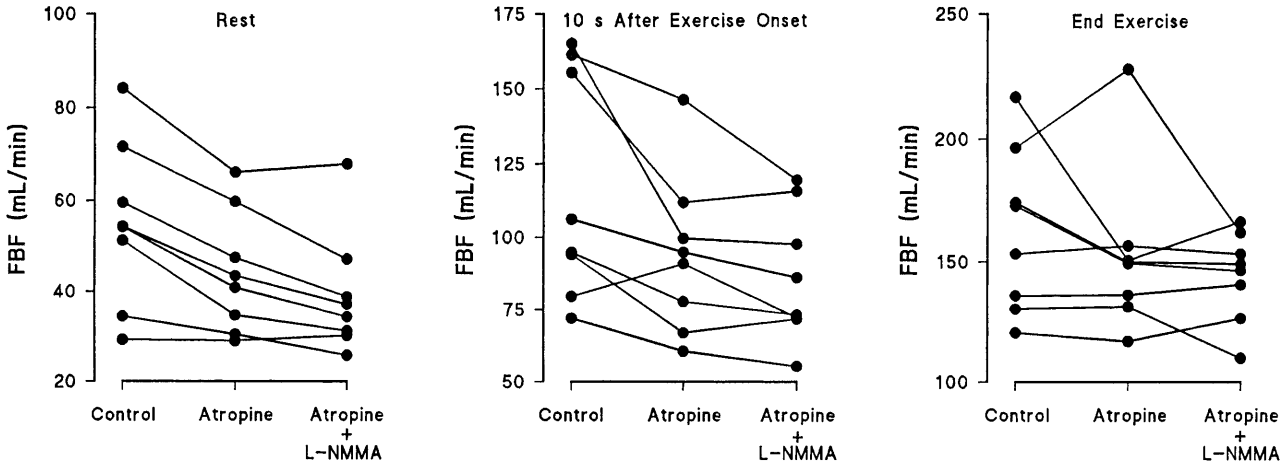

Fig. 3. FBF responses for each subject at rest and after 10 s and 5 min of rhythmic handgrip contractions during control and after local arterial infusions of Atr and subsequent infusions of L-NMMA (Atr + L-NMMA). Consistent reductions in FBF with drug infusions were observed at rest and after 10 s of exercise. Drug effects after 5 min of exercise were more variable.

DISCUSSION

There are several new findings from this study concerning the roles of acetylcholine and NO in regulating the time course of the change in blood flow between rest and exercise. First, the data indicate that both cholinergic and NO mechanisms may modulate blood flow at rest. Second, during exercise and the first 5 s after the cessation of contractions, acetylcholine appeared to contribute to the absolute levels of perfusion. Third, inhibition of NO production in combination with muscarinic blockade blunted the FBF responses during the initial 5 min of recovery, whereas atropine only reduced the peak FBF at 5 s after the cessation of contractions but had little effect on the overall flow response during recovery. By contrast, atropine or L-NMMA given during ongoing contractions appeared to have little impact on the steady-state levels of FBF.

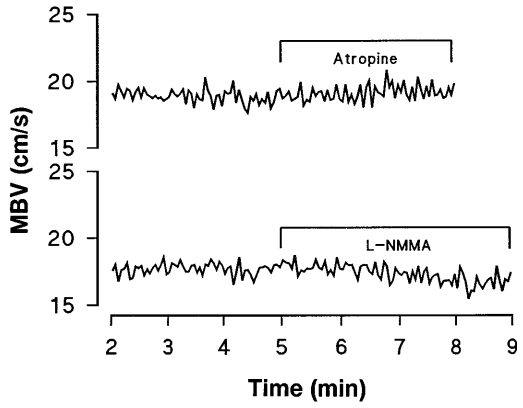

Fig. 4. Infusion of Atr (*top*) or L-NMMA (*bottom*) into brachial artery during steady-state contractions had minimal, if any, effect on exercise mean blood velocity (MBV). Drug infusions began at 5 min of exercise and were continued for 3 min for Atr and 4 min for L-NMMA. Steady-state levels of MBV in *bottom tracing* were lower than in *top tracing* because of Atr infusions given in previous trials. MBV tracings are average of 8 subjects performing 2 repeated trials, and data points are time-averaged over 3-s time intervals.

Limitations

In the current study, we were unable to counterbalance the atropine and atropine + L-NMMA conditions because of the prolonged effects of both drugs. Therefore, the possibility of an interactive effect between acetylcholine and NO mechanisms for blood flow control could not be tested in a randomized manner. Additionally, it is not known whether NO synthase was blocked adequately, because it was not possible to perform the traditional acetylcholine bioassay of NO synthase activation after atropine treatment. However, we used a dose of L-NMMA that has been shown consistently to blunt the vasodilator responses to acetylcholine, suggesting that NO synthase was inhibited (7, 8).

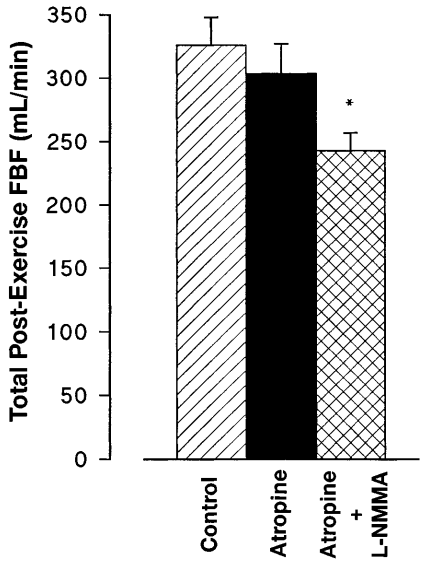

Fig. 5. Total postexercise flow during recovery was calculated as total area under blood flow curve for each experimental condition ($n = 7$). Duration of FBF curve integration was 5 min. * Significantly different from both control and Atr conditions.

Also, we were concerned that the repeated exercise trials might alter the vascular response during the latter trials independent of drug interventions. To address this concern, two subjects performed the experimental protocol without drug infusions. We observed that the FBF at rest before the first and sixth trials (35.6 ± 2.2 and 37.7 ± 1.9 ml/min, respectively) and the end-exercise FBF (156 ± 2 and 167 ± 1 ml/min) were consistent. This suggested that sufficient rest was allowed between trials and that any effect of the repeated trials on the vascular response to the exercise was minimal and tended to be in a direction opposite to that observed after the drug infusions.

Rest

At rest, FBF was progressively reduced from control by the infusions of atropine and then atropine + L-NMMA. Therefore, forearm vascular resistance (MAP/FBF) at rest was increased above control levels by ~19% with atropine and by ~46% with atropine + L-NMMA, if it is assumed that venule pressure at rest was unchanged by the experimental treatments. These changes are in line with previous studies that report a range of increase in resting forearm vascular resistance by ~30% (32) to ~50% (10, 35) after NO inhibition. Such a change in resting vascular resistance might modify the microvascular recruitment with exercise if the vasodilatory stimuli were similar (13). This may explain why muscarinic blockade with atropine and NO inhibition caused an offset in FBF, the magnitude of which was constant between rest and exercise.

On-Transition Phase

The novel aspect of the current study is that the roles of both acetylcholine and NO in modulating the transient blood flow response between rest and exercise were studied. Neither acetylcholine nor NO appeared to regulate the magnitude or rate of increase in the hyperemic response on going from rest to mild rhythmic exercise.

These results differ from those of Armstrong and Laughlin (3), who infused atropine into resting rats and observed no effect on hindlimb blood flow during a subsequent exercise bout. Species-specific vascular responses to acetylcholine may exist. Furthermore, it is likely that drugs infused at rest may not reach the vessels involved in the exercise hyperemia (2, 8). This could explain the results of previous investigators who found an effect of NO synthase inhibition on resting flows in humans but not on the magnitude of the exercise hyperemia (see Ref. 7). In contrast, L-NMMA infused during contractions has been shown to significantly reduce the steady-state exercise blood flow (8, 12).

In light of these previous results, the inability of L-NMMA to alter exercise FBF beyond that produced by atropine in the current study was unexpected. We attempted to maximize the effect of L-NMMA by using mild rhythmic and dynamic exercise of only 5-min duration so that fatigue would be minimized and the recruitment of oxidative muscle fibers would be facilitated (20). In addition, we used an infusion rate of 4 mg L-NMMA/min that has been shown previously to reduce exercise FBF by ~30% (8). However, in the current study exercise FBF was reduced only 10% with both atropine and L-NMMA. In the current study, a downward trend in forearm perfusion was observed when L-NMMA was infused during contractions, but this attenuation of exercise FBF was not as impressive as the reductions reported by Dyke et al. (8). The concentrations of the drug reaching the vascular endothelium involved in the hyperemic response may have been reduced compared with those of the previous study (8), owing to a twofold greater increase in FBF subsequent to the inclusion of hand blood flow and the use of rhythmic isotonic, rather than rhythmic isometric, contractions. Finally, the results may have been influenced by the use of Doppler ultrasound methodology, which allows continuous measures of FBF during the exercise. The only study to demonstrate an effect of L-NMMA on exercise FBF levels in humans (8) used venous occlusion plethysmography. With plethysmographic estimates of FBF, interruption of the exercise is required, resulting in measures that reflect a combination of exercise and postexercise flows where NO inhibition was shown to exert a greater effect in the current study.

Off-Transient Phase

As shown above, the treatment with atropine and then atropine + L-NMMA did not diminish the exercise blood flow response but acetylcholine did appear to adjust the absolute levels of FBF at rest and during contractions. This effect was also observed at 5 s after the cessation of contractions where both atropine and atropine + L-NMMA reduced the peak FBF. However, the overall postexercise hyperemic response was depressed from control only by the combination of atropine and L-NMMA. These results, along with the peak blood flow data after exercise, support the concept that both muscarinic and nonmuscarinic receptor-mediated NO release occurred during recovery.

Role of Acetylcholine

The results of the current study suggest that acetylcholine may be involved in the regulation of the absolute levels of blood flow at rest and during exercise. However, the source of acetylcholine is currently unknown. It is possible that acetylcholine may be released from local neuromuscular junctions with motor unit recruitment (25). However, if this mechanism were the predominant one, the administration of atropine during contractions in this and other studies (1, 19) should have caused at least a temporary reduction in flow. Also, atropine treatment should have had little or no impact on FBF at rest or after contractions if acetylcholine from neuromuscular junctions were important. However, evidence for the existence of both luminal and abluminal muscarinic receptors (23) and for poor diffusion of atropine across the endothelium (15) in arterioles of hamster cheek pouch muscle makes it unclear

whether intra-arterial infusions of atropine block sites at which acetylcholine may act when released from the neuromuscular junction. On the other hand, atropine can cross the blood-brain barrier and, by inference, might also cross the vessel wall. Also, Broten et al. (5) observed that acetylcholine from autonomic cholinergic neurons caused NO release from endothelial cells.

Another possibility is that acetylcholine is released directly from endothelial cells (18) and that this release contributes to the "flow-induced" vasodilation through local activation of muscarinic receptors and release of NO (17). This mechanism would explain the impact of atropine administration at rest and immediately after exercise. Because both acetylcholine- and non-acetylcholine-mediated NO release may contribute to flow-induced dilation, this might also explain why addition of L-NMMA to atropine caused a greater reduction in both resting and postexercise blood flow than atropine alone (17). The lack of effect of either atropine or L-NMMA during contractions may have been caused by the multiple redundant mechanisms that contribute to the steady blood flow responses during exercise, including the muscle pump (14, 27) and other metabolic vasodilators (4, 34). It is also possible that cholinergic autonomic nerves contributed (5), but the existence of this latter mechanism has been disputed (11).

In conclusion, these data suggest that both cholinergic and noncholinergic NO-related mechanisms regulate basal FBF. In addition, the absolute levels of exercise hyperemia were reduced with atropine and atropine + L-NMMA. However, neither acetylcholine nor NO appeared to modulate the magnitude or rate of increase in exercise FBF. In contrast, the total recovery blood flow was reduced with atropine + L-NMMA but not atropine alone, suggesting that NO released by noncholinergic mechanisms contributes to the magnitude of postexercise blood flow.

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