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Role for Nitric Oxide But Not Prostaglandins in Acetylcholine-Induced Relaxation of Rat Cremaster Third-Order Arterioles in 5-Hour Ischemia-Reperfusion Control Rats

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Abstract

Intravital videomicroscopy was used for 1 hr in anesthetized 4- to 5-week-old rats, while mean femoral arterial blood pressure and suffusate Po_2 were continuously monitored. The total duration for experimentation was 5 hr in order to mimic the controls used previously for a 4-hr ischemia and 1 -hr reperfusion model. The specific aim was to examine further the efficacy of this model by (a) assessing the potential role(s) of nitric oxide (NO) and/or prostaglandins (PG) in acetylcholine (ACh)-induced relaxation, and (b) determining if inherently low vasomotor tone (VT) and wall shear stress (WSS) mask latent NO- and/or PG-mediated responses to ACh. Reactivity to 10^{-4} M ACh or 10^{-6} M sodium nitroprusside (NP) were determined in resting third-order arterioles (3A) or in those preconstricted with norepinephrine (10^{-6} M NE) at physiological suffusate Po_2 (25-30 mm Hg). Repeated and randomized topical administrations of ACh, NP, arachidonic acid (10^{-5} M AA), NE, or 10^{-5} M atropine (ATR), N^G-nitro-L-arginine methyl ester (L-NAME), or ibuprofen (Ib) alone or in combination to the surface of exteriorized cremaster flaps, provoked no alteration in mean systemic arterial blood pressure. ACh and NP were equipotent evoking relaxations on the same order of magnitude and duration as reported previously for arterioles with spontaneous or NE-enhanced VT. ATR or L-NAME decreased resting internal diameter by 12 to 14% and reversed relaxation of resting or preconstricted arterioles to ACh but not to NP. Ib failed to elicit blockade. However, administration of AA demonstrated Ib-inhibitable increases of 20 and 52% in resting and NE-preconstricted arterioles, respectively, implicating NO but not PG in the regulation of resting (relaxant) tone and in ACh-induced dilation following activation of 3A muscarinic receptors. The absence of PG-mediated responses appears unrelated to low initial WSS at physiologic suffusate Po_2, since NE-induced elevations of VT and centerline cell velocity also did not cause Ib-inhibitable relaxation. These and our previous findings suggest that the impaired relaxant function of 3A arterioles is caused in part by a paucity in spontaneous tone inherent to this model and by depressed vasoreactivity arising from disruption of NO biosynthesis.

Key Words: **cremaster flaps, arterioles, third order, physiologic suffusate Po_2, vasorelaxation, acetylcholine, nitroprusside, norepinephrine, nitric oxide, prostaglandins, ibuprofen, N^G-nitro-L-arginine methyl ester, atropine**

Introduction

In the field of microsurgery reimplantation of amputated body parts and free tissue transfer have become standard techniques (5). The major concern arising from the utilization of these surgical techniques

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is ischemic injury which is one of the common pathologies causing tissue damage and necrosis. Although some of the factors related to recirculation after ischemia have been investigated (5), the functional alterations occurring to the microvasculature itself from extensive "noflow-reflow phenomenon" are not well understood and require clarification.

There are at least two different mechanisms reported for relaxation of mammalian arteries susceptible to ischemia-reperfusion injury. One first described in 1980 by Furchgott and Zawadski (12) for acetylcholine (ACh) depends upon an intact endothelium's ability to produce and release a relaxant factor (EDRF) following activation of an atropine specific muscarinc receptor. The second one is an endothelium-independent pathway, because it is actuated by nitrogen donors like sodium nitroprusside (NP) exclusive of endothelium and cholinergic receptors.

ACh relaxation is inhibited in numerous vascular beds by denuding vasculature of its endothelium, L-arginine analogues such as N^G-nitro-L-arginine methyl ester (L-NAME) or N^G-monomethyl-L-arginine (L-NMMA), inactivation of vascular smooth muscle soluble guanylate cyclase, and local generation of superoxides (6, 8, 14, 17, 18, 22, 24, 26, 31, 36, 37, 39). Taken together these findings implicate a superoxide degradable nitroso-compound derived from endothelium, possibly nitric oxide (NO), in cholinergic relaxation of vascular smooth muscle.

However, responses to ACh are highly variable among different vascular beds and species, and among vessels within any one vascular bed (4, 6, 11, 15 , 23, 27), indicating that second messengers other than NO trigger endothelium-dependent responses in some preparations (1, 11, 15, 20, 25, 30). In this regard prostaglandins (PG) have been implicated in dilation to ACh or to bradykinin or A23187 (6, 17, 20, 22, 34). Similarly, PG as well as NO are hypothesized to be key mediators of flow-dependent relaxation (l, 9, 11, 13, 15, 20, 21, 25, 26, 34), where increased wall shear stress (WSS) along the luminal surface of endothelium promotes the production and release of EDRF. Since intracellular calcium levels increase (1, 7, 15, 25, 38), some investigators postulate that the molecular inductive pathways for NO and PG are the same for cholinergic and flow-dependent mechanisms. Less clear, however, is the potential interaction between these mechanisms which might facilitate ACh-induced vasodilation by promoting NO and PG synthesis in response to increased WSS.

Interest in this area of research prompted development of a model for studying arteriole responsiveness to ACh and NP in rat cremaster muscle subjected to 4-hour of ischemia and 1- to 5-hour of reperfusion (27). The endothelium-dependent pathway characterized by the action of ACh was blocked by the ischemic insult. Endothelium-independent responses to NP were only slightly reduced when compared to paired controls. It was concluded that ischemia-reperfusion impaired the relaxant function of arterioles without inducing mechanical trauma.

In subsequent studies of 5-hour ischemia-reperfusion control rats, however, both spontaneous tone and vasoreactivity to ACh or NP were diminished independent of the level of oxygenation (25-147 mm Hg) used to equilibrate cremaster flaps (4). These findings conflicted with previous conclusions (27) that vasodepression was unrelated to mechanical trauma from the 5 or more hour postsurgical period used to initiate ischemia-reperfusion injury. Also unresolved were the roles of NO and PG in generating spontaneous VT and ACh relaxation, and of low initial VT and WSS in depressing responses to endothelium-dependent and endothelium-independent relaxants.

Accordingly, in the current study ACh and NP reactivity is re-evaluated and compared to arachidonic acid (AA) responsiveness in rat cremaster 3A arterioles at two levels of initial VT and WSS . The postsurgical period of between 4 and 5 hour and suffusate Po_2 mimic a previous study (4). Blockade of muscarinic receptors, NO synthase (NOS), or cyclo-oxygenase, clarify the endogenous mechanisms underlying vasorelaxation.

Materials and Methods

Surgical and Physiological Procedures

Detailed methods are described elsewhere (4) for the pentobarbital (0.06-0.09 mg/g body weight) anesthetized, 4- to 5-week-old, Sprague-Dawley rats (75-110 g b.w.) subjected to pharmaceutical evaluation between 4-5 h postsurgery.

Drug Protocols

ACh, AA, NP, norepinephrine (NE), ATR, and ibuprofen (Ib), were purchased from Sigma Chemical Company, St. Louis, MO, while L-NAME was obtained from Bachem Bioscience Inc., Philadelphia, PA. Each drug except NE was administered topically by a drip tube attached to the barrel of a 25X water immersion objective centered and focused over each randomly selected 3A arteriole. NE was applied with a syringe at the start and/or completion of experiments. In every case, Ringer's solution served as a carrier. Due to factors associated with dilution and diffusion, the concentrations of these substances and gases at

the surface of a given arteriole were unknown. Therefore, they were expressed as the concentrations applied within the suffusate bathing the surface of the exteriorized cremaster muscle. Po_2, Pco_2, and pH were periodically assessed in the suffusate immediately prior to the start of experimentation using a Model 1306 Instrumentation blood-gas analyzer.

Rats were divided into nine experimental groups of 10 animals each with 10-12 arterioles per group. In preparations with spontaneous (resting) tone, Ringer's solution was administered via the drip tube for two consecutive 3-min intervals during which D and centerline cell velocity (Vcl) were measured as a control. The Ringer's solution (one or two drops) was applied topically with a syringe, and baseline (predrug) values for D and Vcl tracked for an additional 2 min to determine the effect of carrier alone. Then, either ACh (10^{-4} M), NP (10^{-6} M), or AA (10^{-5} M) and one of three potential antagonists (10^{-5} M ATR, L-NAME, or Ib) were randomly applied through the drip tube over the surface of flaps for two consecutive 3-min periods. The drug regiments and concentrations selected were based upon results of previous studies (4, 17, 18, 22, 24, 27). Approximately 10 min was allowed between the paired drug trials to permit recovery to predrug conditions. After recovery, another agonist was tested using the same antagonist over two consecutive 3-min intervals. At the end of the experiment, one or two drops of NE (10^{-6} M) was added topically to the surface of flaps to assess the constrictor integrity of each arteriole.

In arterioles with NE-enhanced tone, the same experimental protocol was followed with two exceptions. First, after the two consecutive administrations of Ringer's solution, one or two drops of NE (10^{-6} M) instead of Ringer's solution was topically applied for 2 min to enhanced initial VT, Vcl, and WSR. This was done prior to ACh or AA plus the specific and selective antagonists. Here again, NE was administered topically at the end of the experiment to reassess the constrictor integrity of the arteriole. Second, NP was not used in conjunction with these antagonists, since in no case did ATR, L-NAME, or Ib block NP-induced dilation of resting arterioles.

Calculations

Data on D and Vcl were digitized on a PC and used to calculate D, VT, mean Vcl (Vm), volumetric flow rate (Q), wall shear rate (WSR), and WSS. The empirical factor of 1.6 was used to convert Vcl to Vm (Vm=Vcl/1.6) (4). Then Vm was multiplied by a system calibration factor to calculate actual mean blood flow velocity. This factor was derived using the IPM calibration wheel (4). Q was determined using Vm ($D^2/4$). WSR was calculated using 8 (Vm/D). Since viscosity (η) offsets changes in WSR from fluctuations in Hct accompanying vasomotion, WSS also was computed using WSR x η (28). VT (%) was determined from $(D_{bl}-D_{max})/D_{bl}\times100$, where D_{bl} represents resting or NE-enhanced baseline diameter and D_{max} the maximum change in D produced by each agonists/antagonist combination. The percent changes from control for D, Vm, Q, or WSR were derived using $(Nx-Nc/Nc)\times100$, where Nc is the pre-drug (control) baseline for each parameter (N) and Nx is the value obtained with each concentration of drug alone or in combination.

Statistics

Means (±SE) for D, VT, Vm, Q, and WSR were computed for 60-second baseline values, maximal responses from baseline, and recovery. They were compared among control and experimental groups at the 95% confidence level using analysis of variance (ANOVA). When ANOVA results were significant, groups were compared using Tukey's test. Data were reported as percentage increase or decrease from baseline, or normalized as a percentage of the 60-second mean baseline. In addition, calculated values for WSS and viscosity were determined from measurements of D and Vm (28). Linear regression analysis was used to evaluate the temporal relationships between either ACh and ATR or ACh and L-NAME. The t-test was used to determine significance between response slopes for D. Finally, since maximal responses for both D and Vcl occurred between 50 to 180 s post-drug, mean values (±SE) for D, Vm, WSR, WSS, Q, and VT were computed for this duration.

Results

In no case did Ringer's solution, or any of the agonist antagonist combinations, induce a significant change in mean systemic arterial blood pressure (80-112 mm Hg) or core body temperature (35-36°C) after topical administration to the surface of cremaster flaps. No skeletal muscle twitching, vasospasm, or other microcirculatory disturbances followed topical application of the carrier Ringer's solution (27) when suffusate Po_2 was maintained at 25-30 mm Hg (4).

Arteriolar Responses with Resting Tone

The mean (±SE) D for 3A arterioles with resting tone was 23.6 (±2.6) μm. When given alone, ACh (10^{-4} M) caused significant increases in D of approximately 21% over baseline in agreement with

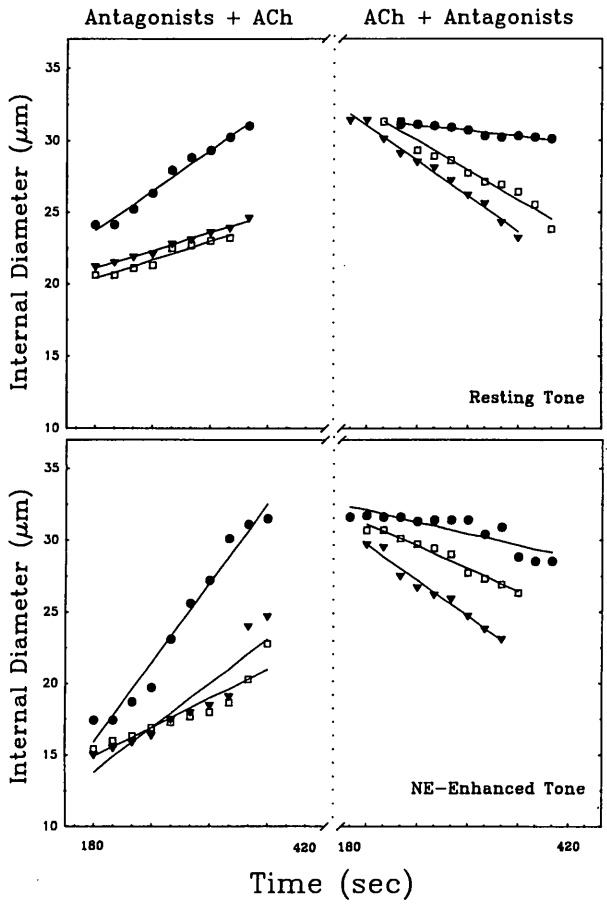

Fig. 1. Linear regression analysis of D versus time demonstrating blockade of 10^{-4} M ACh (●)-induced dilation by pre- and post-treatments with 10^{-5} M ATR (❑) or with 10^{-5} M L-NAME (▼) in rat cremaster 3A arterioles with resting or 10^{-6} M NE-enhanced tone. Ringer's solution (R) was used as a carrier for each drug. (a). =p<0.05 vs. R+ACh; (b). =p<0.05 vs. ACh +R. n=10 each.

Experimental group	Slope±SE	
	Resting tone	NE-enhanced tone
R+ACh	0.047±0.003	0.092±0.006
ATR±ACh	0.022±0.002[a]	0.034±0.005[a]
L-NAME+ACh	0.021±0.001[a]	0.051±0.008[a]
ACh+R	-0.006±0.001	-0.014±0.003
ACh+ATR	-0.034±0.002[b]	-0.026±0.002[b]
ACh+L-NAME	-0.041±0.002[b]	-0.042±0.003[b]

previous research (4). Peak dilation occurred 50 to 90s after application of ACh and returned to predrug values by 250-300 s after maximal dilation. In contrast, Vm decreased by 14-20% and WSR by 22-30% from resting baselines of 2.0-2.2 mm/s and 663-740 s^{-1}, respectively. Despite the significant changes in D and Vm, ACh failed to elicit an increase in the resting values for Q (598-621 pl/s).

ATR and L-NAME, but not Ib, when administered alone significantly decreased D from the resting mean by 12 and 14%, respectively.

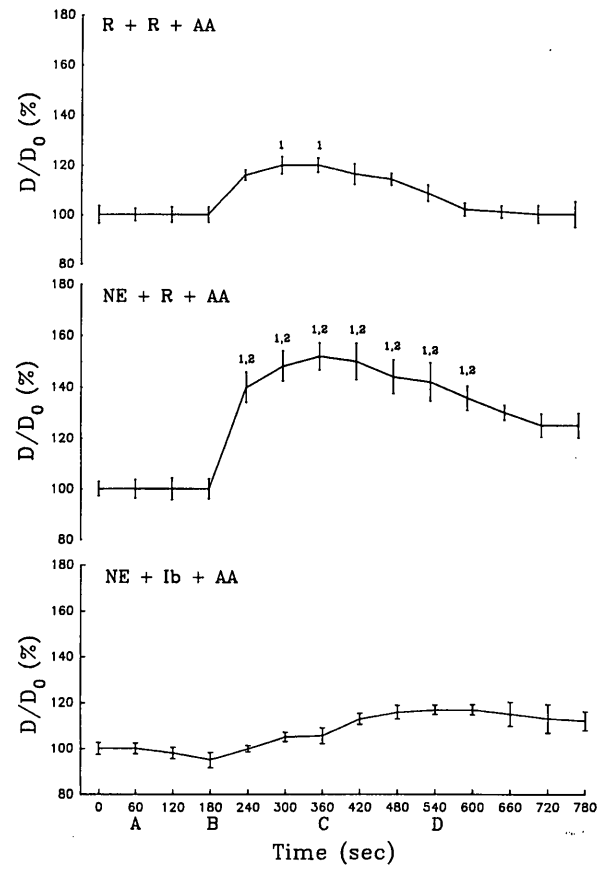

Fig. 2. The effect of 10^{-5} M Ib pre-treatment on 10^{-5} M AA-induced dilation of 3A arterioles with resting or 10^{-6} M NE-enhanced vascular tone. D/Do = the normalized percentage of the mean 60-second baseline for this parameter; 1=p<0.05 vs. resting baseline; 2=p<0.05 vs. NE+Ib+AA; (A). = topical application of Ringer's solution (R) or Ib alone; (B). = topical application of AA in combination with R or Ib pre-treatment; (C). = topical reapplication of AA alone; (D). topical readministration of R. n=10 each.

Maximal vasoconstriction occurred within 60 s after the application of ATR or L-NAME with recovery approximately 90 s after cessation of blockers. However, there were no significant changes in Vm, Q, or WSR to either antagonist.

Repeated and randomized applications of ATR or L-NAME significantly attenuated ACh-induced dilation by 17-20% and 14-18%, respectively, from peak relaxations of 29.4 and 29.0 μm. This inhibitory relationship was illustrated using linear regression analysis of the temporal changes in D (Fig. 1, upper panel). The slopes for arteriolar dilation were severely depressed and for recovery significantly facilitated by ATR or L-NAME pre- and post-treatments, respectively. With one exception, Vm, WSR, and Q did not differ significantly after blockade from ACh alone; viz., Vm and WSR were both significantly elevated in only the L-NAME post-treatment group when compared to ACh alone (1.9 and 524 to 3.1 mm/s and 1008 s^{-1}, respectively).

Ib had no effect on ACh-induced responses. Yet, a significant Ib-inhibitable relaxation to AA of 20% over the 23.3±2.9 μm resting mean (baseline) D was demonstrated in 3A arterioles (Fig. 2, top panel). In all cases, maximal responses to AA occurred within 240-300 s and returned to predrug baseline 290-360 s later. Vm significantly decreased by 25% and WSR by 33% from resting baselines of 1.6 mm/s and 549 s^{-1}, respectively. Responses occurred in approximately 55 s and returned to control values in about 380 s. However, there was no significant alteration in the mean resting value for Q (430±91 pl/s).

As in a previous study (4), topical application of NP (10^{-6} M) provoked significant relaxation of approximately 26% from a mean of 23.3-25.5 μm to peak values of 29.5-31.8 μm (data not shown). Recovery occurred 320 s after maximal dilation. Vm significantly decreased by 17-29% from resting values of 1.7-2.0 mm/s reaching peak response by 90 s and recovery by 270 s. WSR decreased by 29-36% from between 565-646 s^{-1} to 377-432 s^{-1}. In contrast, increases in Q of 15-31% from resting values of 446-581 pl/s were not significant.

In no case did ATR, L-NAME, or Ib block NP-induced changes in D, Vm, WSR, or Q. Therefore, NP was not used in conjunction with these antagonists in NE-preconstricted arterioles.

Arteriolar Responses with NE-Enhanced Tone

Topical applications of NE (10^{-6} M) caused immediate and significant constriction of 3A arterioles at the inception and completion of each experiment. There was no escape from NE-induced arteriolar constriction for the 2 min preceding the administration of agonists and/or antagonists or for up to 5 min following NE alone (4). In 46 trials, D decreased by 20-31% from between 23.3-25.1 μm to 16.2-19.0 μm. Vm significantly increased by 11-55% from a control Vm of 1.1-1.9 mm/s to 1.7-2.1 mm/s, and WSR significantly rose by 46-95% from a control WSR of 366-671s^{-1} to 715-977 s^{-1}. Likewise VT and WSS increased between 20-31% and 35-67%, respectively, from resting values. In contrast, Q decreased 21-47% from baselines of 430-514 pl/s to 273-341 pl/s. The NE-induced responses were not modified by ATR, L-NAME, or Ib pre- or post-treatments.

ACh induced significant relaxation of 56-74% in NE-preconstricted arterioles (Fig. 3) from 16.2-19.0 μm to 28.2-29.7 μm. However, there were no significant changes in the time to maximal response or recovery. As in previous research (4), the increases in D were 2.6-3.5 fold greater than in arterioles with resting VT. Maximal responses occurred within 110 s and returned to pre-NE values 230-320 s after peak

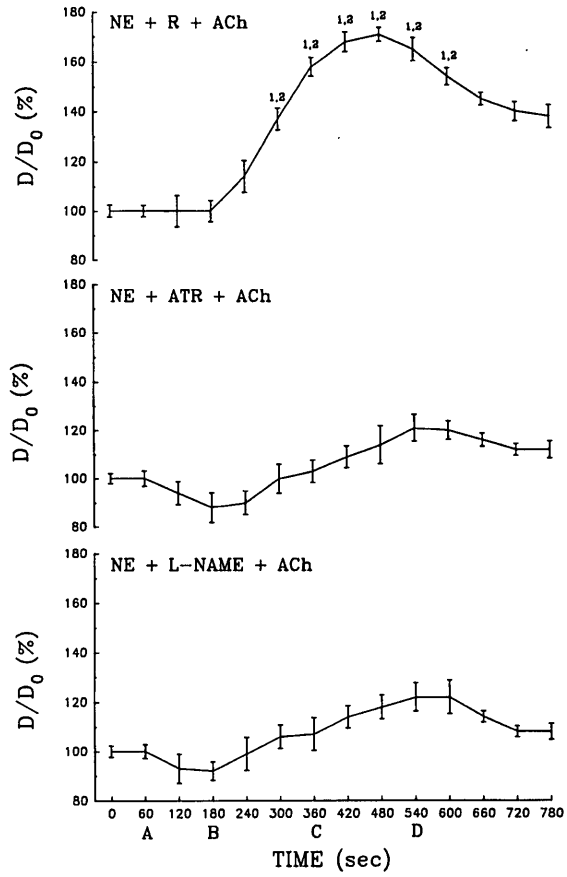

Fig. 3. The effect of 10^{-5} M ATR or 10^{-5} M L-NAME pre-treatment on 10^{-4} M ACh-induced dilation of 3A arterioles with 10^{-6} M NE-enhanced tone. D/Do=the normalized percentage of the mean 60-sec baseline for this parameter; 1=p<0.05 vs. NE-enhanced baseline; 2=p<0.05 vs. NE+ATR+ACh or p<0.05 vs. NE+L-NAME+ACh; (A). = topical application of Ringer's solution (R), ATR, or L-NAME alone; (B). = topical application of ACh in combination with R, ATR, or L-NAME pre-treatment; (C). = topical reapplication of ACh alone; (D). topical readministration of R. n=10 each.

relaxation. Vm significantly decreased by 18-24% from base line values of 1.7-2.1 mm/s and reached peak response by 90 s and recovery by 270 s. WSR also significantly decreased 43-54% from baselines of between 715-977 s^{-1} to 382-454 s^{-1}, while Q significantly increased by 88-132% from 273-312 pl/s to 587-633 pl/s. These increases were 5 and 7 fold greater than in arterioles with resting tone.

Topical administration of either ATR or L-NAME elicited no additional constriction of arterioles with NE-enhanced tone. However, ACh-induced dilation was significantly attenuated by 35-59% (Fig. 3) from 28.2-29.3 μm to 19-23.2 μm with ATR and to 18.6-19.2 μm with L-NAME. This inhibitory relationship (Fig. 1, lower panel) was like that seen with resting tone (Fig. 1, upper panel). ATR significantly blunted decreases in Vm by 17% and in WSR by 22% post-treatment and 40% pre-treatment.

L-NAME also significantly blocked the fall in WSR by 11% post-treatment and 29% pre-treatment. Although L-NAME pre-treatment had no significant impact on Vm, post-treatment suppressed Vm by an additional 15% over ACh alone. The rise in Q was significantly depressed by 42% for ATR post-treatment, 97% for ATR pre-treatment, and 128-146% for pre- or post-treatment with L-NAME. Ib did not modify ACh-induced responses.

AA induced approximately a 52% increase in D (Fig. 2, middle panel) from 18.6 to 28.2 μm; this response was about 2.5 fold greater than in arterioles with resting VT. As in resting arterioles, peak dilation occurred at 100 s with recovery to pre-NE (Ringer's) baseline approximately 300 s after maximal vasorelaxation. Although WSR was significantly depressed by 44% from a NE-enhanced baseline of 840 s^{-1}, there were no significant decreases in Vm from baseline. However, Q significantly increased by 86% from 341 to 635 pl/s. For all parameters examined except Q, there were no changes in the times to maximal response and recovery for AA in NE-preconstricted arterioles. However, the increase in Q was 110 s earlier than in 3A arterioles with resting tone.

Ib significantly attenuated AA-induced dilation by 38-40% (Fig . 2, lower panel) from a peak relaxation of 28.2 μm. Ib also blunted increases in Q by 82-91% to 324-355 pl/s. However, Ib had no effect on either WSR (606-664 s^{-1}) or Vm (1.6-1.7 mm/s).

Discussion

The control for a 4-5 hour ischemic model of rat cremaster muscle (4, 27) was examined for 1 hour with intravital microscopy to study ACh-induced relaxation at two levels of initial vasomotor tone (VT) and a physiologic suffusate oxygen tension (25-30 mm Hg). The influence of NE-enhanced initial VT and WSR on ACh responses was evaluated alone and in combination with pre-and post-treatments of specific and selective muscarinic (ATR), NOS (L-NAME), or cyclo-oxygenase (Ib) antagonists. This approach included cross-blockade of reported endothelium-independent (NP) or other endothelium-dependent (AA) vasodilators.

Results of the current study support the existence of endogenous NO and PG relaxant pathways in our 5-hour ischemia-reperfusion control rats. They also agree with Koller and Kaley (17, 18, 22, 24) that NO but not PG control cholinergic vasorelaxation following activation of muscarinic receptors, but conflict with de Wit et al. (6) that NO and PG both mediate spontaneous tone and ACh relaxation. Absence of PG-mediated responses appeared unrelated to low initial WSR, since elevation of initial VT and Vm with NE did not trigger Ib-inhibitable relaxation. ACh or AA was equipotent to the NOS- and cyclo-oxygenase-independent agent NP. Moreover, both ATR or L-NAME (but not Ib) alone constricted resting but not NE-preconstricted arterioles suggesting a role for muscarinic receptors and basal levels of NO in the regulation of physiologic vascular tone. These findings validate our model, and the protocols used to assess reactivity and blockade, by demonstrating that 3A arterioles were functionally intact and that vasodilation to ACh or AA and not NP was specifically and selectively modified by exogenous application of one or more antagonist(s).

The enhanced responsiveness of preconstricted arterioles is attributed to NE resetting the predrug baseline for D. This conclusion was based upon the finding that D following peak relaxation reached the same diameter in resting or NE- or O_2-preconstricted arterioles (4, 23, 27) and followed the same time course to maximal response and recovery. Although preconstriction accelerated the time to Qmax, absolute peak values again did not differ significantly from resting arterioles (4).

The inhibitory effects of ATR, L-NAME, and Ib on Vm, WSR, or Q were highly variable regardless of the level of initial VT. These findings were in agreement with a previous study (4) where similar variations were seen after topical application of ACh or NP alone. However, D was a reliable measure of reactivity in arterioles at both levels of tone. Inhibition was reproducible for Q only when initial VT and WSR were amplified for 2 min by NE pre-treatment. The use of a physiologic suffusate Po_2 complemented this approach by blocking the potential adverse effect of adrenergic escape on initial VT and WSR (4).

ATR or L-NAME modified ACh relaxation in resting and NE-preconstricted arterioles. Paired repeated and randomized treatments both attenuated and delayed vasorelaxation. They also accelerated the time to recovery to predrug (Ringer's) values, when each antagonist was given before and after peak response verifying affinity for muscarinic receptors or NOS, respectively. The results using ATR or L-NAME alone, or in combination with ACh, implicated NO in the regulation of spontaneous tone and in 3A arteriole relaxation following activation of muscarinic receptors. They also suggest that the impaired relaxant function of arterioles in ischemia-reperfusion control rate, and presumably ischemia-reperfusion injury (27), is caused at least in part by a paucity in spontaneous tone inherent to this model (4), and by depressed vasoreactivity arising from disruption of NO biosynthesis. The sites of muscarinic receptor localization and NO production though undefined in the current study are being determined within our 5-hour ischemia-reperfusion control rats. Experimentation

also includes characterization of the apparent role of endogenous ACh in the control of basal tone via NO release, especially since numerous reports indicate that rat skeletal muscle microvasculature is devoid of direct cholinergic sympathetic innervation (3, 10).

The fact that ATR or L-NAME alone produced constriction of resting arterioles also raised unresolved questions as to whether specific and selective blockade, or some indiscriminate mechanism(s), attenuate(s) ACh-induced dilation. Although L-NMMA and other NOS inhibitors provoke arteriolar constriction in addition to ameliorating ACh-induced relaxation (11, 16, 17, 24, 30, 31, 36), ATR is vasoinactive (6, 12, 24). A nonselective inhibition might be explained by arteriolar preconstriction decreasing the relative relaxation to ACh either by lowering the pre-ACh baseline for D, or by a change in vascular wall length tension relationships. In this regard, Klitzman et al. (19) demonstrated that adrenergically mediated increases in the contractile state of arterioles (or some unidentified metabolite) blunt the action of endogenous (relaxant) metabolites by an as yet undefined mechanism. Such concerns in the current experimental design resulted in blockade being evaluated in NE-preconstricted arterioles where relaxation to ACh, NP, or AA is heightened and neither ATR, L-NAME, nor Ib is vasoactive.

Another potential contraindication for using NOS inhibitors is that they amplify the predominant α_2 constrictor effect of exogenous NE. Nakamura and Prewitt (32) attribute amplification to NOS blockade of endothelial α_2 receptor-facilitated NO synthesis and release. Ohyanagi et al. (33) ascribe it to interference between NE and tonicly released EDRF at the smooth muscle of arterioles which promotes α_2 mediated constriction during NOS inhibition. However, in our preconstricted preparations, L-NAME never provoked significant enhancement of peak constriction to exogenous NE. This was true despite L-NAME or L-NMMA (32, 33) consistently constricting arterioles with spontaneous tone, and antagonizing ACh-induced dilation of resting and NE-preconstricted arterioles. Moreover, the lack of adrenergic escape for 2 min before NOS blockade, and for up to 5 min after NE, served as an indicator of adequate suffusate and tissue oxygenation (2, 6, 32).

Although a PG-mediated relaxant pathway exists in our 5-hour cremaster preparations, it was not a factor in the generation of spontaneous tone or in cholinergic (muscarinic) mechanisms. Yet, prostaglandins play a role in the regulation of resting vascular tone and in flow-dependent vasodilation within numerous vascular beds (1, 9, 11, 13, 15, 20, 21, 25). Indomethacin or meclofenamate attenuates peak relaxation following augmentation of WSS (20, 21, 25), and the levels of PG correlate with increases in rabbit femoral artery (15). A six- to seven-fold enhancement of WSS accompanies vasoconstriction at a constant flow rate or increased flow at a constant diameter. This elevation provokes an 11- to 12-fold elevation of NO and prostacyclin in endothelial cells.

Despite NE-induced increases in initial WSR of 46-95% and initial WSS of 35-67% in the current study, Ib failed to elicit vasoconstriction or to attenuate ACh dilation. These findings agreed with Koller and Kaley (17, 18, 20, 21) where room air, rather than NE at physiological suffusate Po_2, was used to enhance arteriole VT and WSR (104%). In contrast, a PG response to increased WSS has been demonstrated by Hecker et al. (15). In this investigation, however, WSS was enhanced throughout the entire experimental period and not just for 2 min as in our model. Therefore, longer durations may be required for activation of cyclo-oxygenase by flow-dependent mechanisms even though tissue hyperoxygenation with room air which increases VT and WSR failed to elicit induction of the PG relaxant pathway (15 ,20, 21, 25). This too might be explained by tissue hyperoxygenation blunting or inhibiting PG biosynthesis (14, 35, 37).

Therefore, further research is required to definitively assess the relationship between WSS and VT. Such investigations would benefit from (a). the use of more physiological dosages of ACh, AA, NP, and/or NE, (b). sustained increases in WSS using either O_2 or parallel arteriole occlusion (18, 20-23), and (c). application of altemative blockers and contemporary cellular and molecular methods. These studies might also re-examine this relationship in atherosclerosis or in ischemia-reperfusion injury at experimental durations less than 4- to 5-hour postsurgery, since these disease states damage the vascular wall (40), and in ischemia also appear to inhibit NO biosynthesis (27).

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