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Acetylcholine and sodium nitroprusside cause long-term inhibition of EDCF-mediated contractions

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Tang, Eva H. C., Michel Feletou, Yu Huang, Ricky Y. K. Man, and Paul M. Vanhoutte. Acetylcholine and sodium nitroprusside cause long-term inhibition of EDCF-mediated contractions. *Am J Physiol Heart Circ Physiol* 289: H2434–H2440, 2005. First published July 22, 2005; doi:10.1152/ajpheart.00568.2005.—Preliminary studies suggested that previous exposure to acetylcholine (ACh) exerts a delayed inhibition of subsequent contractions mediated by endothelium-derived contracting factor (EDCF). To confirm this long-term inhibitory effect of ACh and to determine whether nitric oxide (NO) mediates the phenomenon, we suspended rings of spontaneously hypertensive rat (SHR) aortas in organ chambers for the recording of isometric force. The rings were incubated in the absence or presence of N^{ω}-nitro-L-arginine methyl ester (L-NAME; inhibitor of NO synthases) or 1*H*-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (ODQ; inhibitor of soluble guanylyl cyclase) before exposure to increasing concentrations of ACh or sodium nitroprusside (SNP) during contractions to phenylephrine. Thereafter, EDCF-mediated contractions to ACh or the calcium ionophore A-23187 were elicited. If the rings were preexposed to ACh or SNP, the subsequent ACh-induced EDCF-mediated contractions were reduced compared with those obtained in rings of the same arteries not previously exposed to either agent. ODQ did not affect the inhibition caused by preexposure to ACh but significantly reduced that caused by preexposure to SNP. Previous exposure to SNP reduced, whereas previous exposure to ACh did not affect, endothelium-dependent contractions to A-23187. Previous exposure to either ACh or SNP did not affect the contractions to the thromboxane mimetic U-46619. Thus ACh and SNP exert delayed inhibition of EDCF-mediated contractions via distinct pathways. The effect of ACh is NO independent and upstream of the increase in calcium concentration that triggers the release of EDCF. The effect of SNP is downstream of the calcium rise and is mainly NO dependent.

endothelium-dependent contractions; endothelium-derived contracting factors; spontaneously hypertensive rats; nitric oxide

ACETYLCHOLINE (ACh) and other neurohormonal mediators evoke the simultaneous release of endothelium-derived relaxing factors (EDRF, in particular nitric oxide) and of endothelium-derived contracting factors (EDCF) to control the tone of the underlying vascular smooth muscle (10, 16, 23). However, as an artery ages or undergoes chronic hypertensive stress, the balance between EDRF and EDCF becomes dysfunctional to favor the production of EDCF (16, 17, 32). In particular, EDCF-mediated contractions are prominent in the aorta of spontaneously hypertensive rats (SHR) (17, 38), but they also occur in arteries of other species (14, 20, 29, 30).

Endothelium-dependent contractions are mediated by products of cyclooxygenases (17, 32). The cyclooxygenases metabolize arachidonic acid into endoperoxides, which can be further broken down by enzymes forming prostaglandin E_2, prostaglandin D_2, prostaglandin $F_{2\alpha}$, prostacyclin, and thromboxane A_2 (9). The precise prostaglandin accounting for EDCF is uncertain. One or several of these prostanoids can diffuse across to the smooth muscle and activate thromboxane-prostanoid (TP) receptors, which then leads to an influx of calcium into the smooth muscle cells and causes its contraction (12, 32).

Nitric oxide acutely inhibits EDCF-mediated responses, because these contractions can be augmented in the aorta of SHR when inhibitors of nitric oxide are added to the preparations (1, 37). The same can be achieved by using inhibitors of soluble guanylyl cyclase, such as 1*H*-[1,2,4]-oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) (37). Therefore, the acute effects of nitric oxide are not limited to the chemical inactivation of EDCF but also comprise stimulation of soluble guanylyl cyclase (1). The ability of nitric oxide to scavenge oxygen-derived free radicals, which are essential for the production of EDCF also, could contribute to inhibition of EDCF formation (36). These inhibitory effects of nitric oxide justify the addition of nitric oxide synthase or guanylyl cyclase inhibitors to optimize endothelium-dependent contractions (1, 19, 22, 36, 38).

Preliminary observations in the SHR aorta yielded unexpected results in that, if early in the experiments the presence of endothelium was tested using increasing concentrations of ACh (10^{-10} to 10^{-4} M, during contractions to phenylephrine), subsequent EDCF-mediated contractions were minimal (Fig. 1). This finding suggested that previous exposure to ACh resulted in prolonged inhibition of either the production or the actions of EDCF. The present studies were designed to substantiate these preliminary observations and to determine whether or not nitric oxide is the sole mediator of this long-lasting inhibition of endothelium-dependent contractions. The role of soluble guanylyl cyclase was also examined.

METHODS

Tissue preparation. The experiments were performed on aortas of 38- to 48-wk-old male SHR (~380–460 g) purchased from the Chinese University of Hong Kong and raised at the University of Hong Kong. All experimental protocols were approved by the Institutional Animal Care Committee. The animals were housed in a

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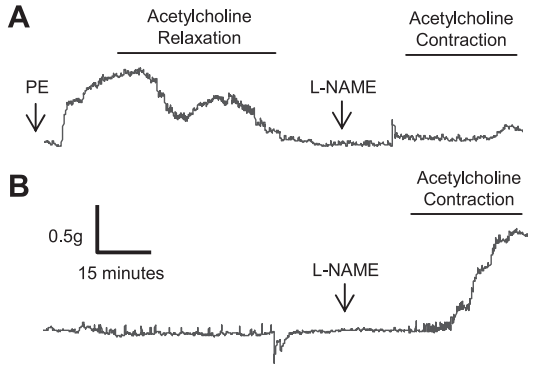

Fig. 1. Tracings from organ chamber experiments showing contractions by acetylcholine (ACh; 10^{-8} to 10^{-5} M) in rings with (*A*) or without (*B*) previous exposure to ACh (10^{-10} to 10^{-4} M). N^{ω}-nitro-L-arginine methyl ester (L-NAME; 10^{-4} M) was added to the preparations before induction of contractions to ACh. PE, phenylephrine.

temperature-controlled room (21 ± 1°C) with a 12:12-h light-dark cycle (0700 lights on, 1900 lights off). Animals had free access to chow (LabDiet 5010) and water. The rats were anesthetized with pentobarbitone sodium (70 mg·ml^{-1}·kg^{-1} ip), and the aorta was then dissected free, excised, and placed in cold, modified Krebs-Ringer bicarbonate solution of the following composition (in mM): 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, and 11.1 glucose (control solution). The blood vessels were cut into rings of 3–4 mm in length. In some preparations, the endothelium was removed mechanically by inserting the tip of a syringe needle into the ring and rolling them back and forth in a Sylgard-based container filled with control solution. The rings were suspended in organ chambers, which contained control solution (37°C), aerated with 95% O_2 and 5% CO_2. They were connected to force transducers (FT03; Grass Instrument, Quincy, MA) for isometric tension recording (Data Logger, Pico Technology, Cambridge, UK). The rings were allowed to equilibrate for an hour at their optimal resting tension of ~2.5 g, as determined in preliminary experiments. All changes in tension were expressed as percentages of the reference contraction to 60 mM KCl obtained at the start of the experiment.

Acetylcholine. In certain rings, the presence of the endothelium was verified by using cumulative concentrations of ACh (10^{-10} to 10^{-4} M) during contractions to phenylephrine (2×10^{-7} M) (preexposure to ACh). The rings were then washed thoroughly, incubated with N^{ω}-nitro-L-arginine methyl ester (L-NAME, a nitric oxide synthase inhibitor; 10^{-4} M), and exposed further to progressively increasing concentrations of ACh (10^{-8} to 10^{-5} M) or the calcium ionophore A-23187 (10^{-8} to 3×10^{-6} M) in the absence of phenylephrine to elicit endothelium-dependent contractions. In some preparations, the rings were incubated with L-NAME and then endothelium-dependent contractions to ACh or the calcium ionophore A-23187 were obtained directly (no preexposure to ACh). Rings with endothelium treated with either S-18886 [selective TP receptor antagonist; 10^{-7} M (27)] or indomethacin [nonspecific inhibitor of cyclooxygenases; 10^{-5} M (17)] and rings without endothelium were studied in parallel. Drug incubation time was 40 min.

Sodium nitroprusside. Rings with endothelium were exposed to the nitric oxide donor sodium nitroprusside (SNP; 10^{-10} to 10^{-4} M) to obtain concentration-relaxation curves during contractions to phenylephrine. The total exposure to SNP was 40 min. The ability of increasing concentrations of ACh or the calcium ionophore A-23187 to produce endothelium-dependent contractions in the presence of L-NAME was then determined.

Thrombin. Rings with endothelium were exposed to a single dose of thrombin (0.0001–10 U/ml) during contractions to phenylephrine for a duration of 40 min before the incubation of L-NAME, and the ability of increasing concentrations of ACh to produce subsequent endothelium-dependent contractions was determined.

Concentration dependency of inhibition of endothelium-dependent contractions. Rings with endothelium were exposed to different ranges of ACh or SNP concentration (from 10^{-10} to 10^{-4} M) during contractions to phenylephrine. The total time of exposure to ACh or SNP was kept at 40 min. The preparations were then washed and incubated with L-NAME, and the ability of increasing concentrations of ACh to produce endothelium-dependent contractions was determined.

Exposure-time dependency of inhibition of endothelium-dependent contractions. Rings with endothelium were exposed to either ACh (10^{-4} M) or SNP (10^{-4} M) for 5, 20, or 40 min during contractions to phenylephrine. The preparations were washed after the preexposure treatment and incubated with L-NAME. The ability of the rings to produce endothelium-dependent contractions to ACh was determined.

Role of nitric oxide. The ability of ACh or the calcium ionophore A-23187 to evoke endothelium-dependent contractions was tested in rings incubated with either L-NAME (10^{-4} M) or ODQ (10^{-5} M) given before the initial preexposure to ACh or SNP.

TP receptor sensitivity. Contractions to increasing concentrations of the thromboxane mimetic U-46619 (10^{-10} to 10^{-6} M) were compared in rings previously exposed to either ACh or SNP.

Muscarinic receptor sensitivity. Rings were contracted with phenylephrine and exposed to increasing concentrations of ACh. After washout and 40 min of equilibration, a concentration-relaxation curve to ACh was obtained during contractions to phenylephrine.

Drugs. ODQ, ACh, the calcium ionophore A-23187, indomethacin, L-NAME, phenylephrine, SNP, and thrombin were purchased from Sigma Chemical (St. Louis, MO). U-46619 was purchased from Biomol (Plymouth Meeting, PA). 3-[(6-Amino-4-chlorobenzensulfonyl-2-methyl-5,6,7,8-tetrahydronaphth)-1-yl] propionic acid (S-18886) was a kind gift from the Institut de Recherches Servier (Suresnes, France). A stock solution of calcium ionophore A-23187 was prepared in absolute DMSO. A stock solution of indomethacin was prepared in a 5×10^{-3} M sodium bicarbonate solution. A stock solution of U-46619 was prepared in ethanol. All other compounds were prepared in deionized water. Concentrations are expressed as final molar concentrations in the bath solution.

Data analysis. Results are presented as group means ± SE, with *n* representing the number of individual observations. The effect of drugs or preexposures on the responses to the agonists was analyzed

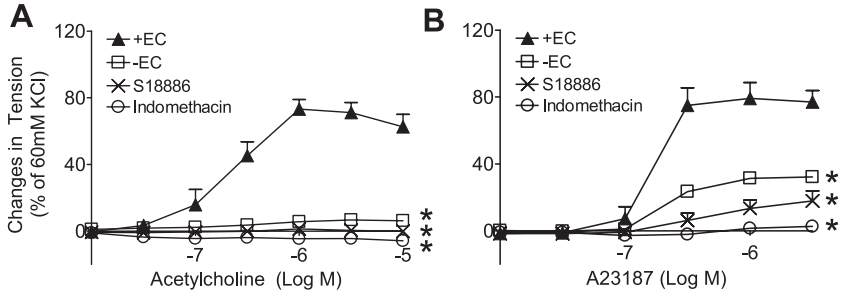

Fig. 2. Contractions to increasing concentrations of ACh (n = 4–12) (*A*) or the calcium ionophore A-23187 (n = 5) (*B*) in rings of rat aorta with (+EC) or without endothelium (−EC) in the absence or presence of either S-18886 (10^{-7} M) or indomethacin (10^{-5} M). Data are expressed as means ± SE. *$P < 0.05$ vs. +EC.

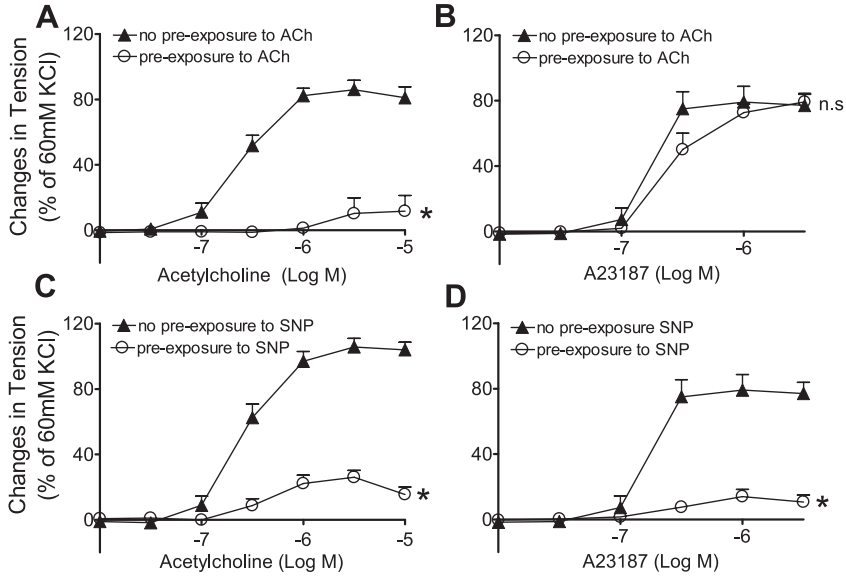

Fig. 3. Contractions to increasing concentrations of ACh ($n = 12$) (*A*) or the calcium ionophore A-23187 ($n = 6$) (*B*) in rings with endothelium of rat aorta are shown with or without previous exposure to ACh (10^{-10} to 10^{-4} M). Contractions to increasing concentrations of ACh ($n = 8$) (*C*) or calcium ionophore A-23187 ($n = 4$–5) (*D*) in rings with endothelium of rat aorta are shown with or without previous exposure to sodium nitroprusside (SNP; 10^{-10} to 10^{-4} M). Data are expressed as means ± SE. *$P < 0.05$ vs. no previous exposure to ACh or SNP.

by means of the area under the dose-response curve. Statistical analysis was performed using Student's *t*-test for control and treatment comparisons and by using ANOVA for analysis of multiple comparisons, followed by Tukey's multiple comparison test, where appropriate, using Prism (version 3a; GraphPad Software, San Diego, CA). A difference was accepted as statistically significant when probability (*P*) values were <0.05.

RESULTS

Endothelium-dependent contractions. Acetylcholine and the calcium ionophore A-23187 evoked contractions in rings with endothelium, which had not been previously exposed to either ACh or SNP and were incubated with L-NAME. These contractions were not seen in rings without endothelium and were abolished by indomethacin or S-18886 (Fig. 2). Acetylcholine produced a biphasic contraction. Between 10^{-8} and 10^{-6} M, endothelium-dependent contractions progressed in a concentration-dependent manner. However, at doses of 3×10^{-6} M and higher, they progressively declined (Fig. 2). The contractions elicited by A-23187 were sustained (Fig. 2).

Preexposure to ACh and SNP. If at the beginning of the experiment a concentration-relaxation curve to ACh was obtained during contractions to phenylephrine, the subsequent endothelium-dependent contraction to ACh was inhibited significantly compared with rings of the same arteries not previously exposed to the muscarinic agonist (Fig. 3*A*). The endothelium-dependent contractions to the calcium ionophore A-23187 were not significantly different whether rings were or were not previously exposed to ACh during contractions to phenylephrine (Fig. 3*B*). If at the beginning of the experiment a concentration-relaxation curve to SNP was obtained during contractions to phenylephrine, the subsequent endothelium-dependent contractions to both ACh (Fig. 3*C*) and the calcium ionophore A-23187 (Fig. 3*D*) were inhibited significantly compared with rings of the same arteries not previously exposed to the nitric oxide donor.

Dose-dependent inhibition by ACh. Acetylcholine affected contractions to phenylephrine in a triphasic manner. A concentration-dependent relaxation was achieved from 10^{-10} to 10^{-7} M. This was followed by a rebound in tension at concentrations between 10^{-7} and 3×10^{-6} M. The third phase was a secondary fall in tension from 3×10^{-6} M onward. Treatment with ODQ or L-NAME prevented the relaxations to ACh (Fig. 4*A*). Previous exposure to different ranges of ACh concentrations inhibited subsequent EDCF-mediated responses in a concentration-dependent manner (Fig. 4*B*). The greatest inhibition was observed when the rings were exposed previously to high doses of ACh (up to 10^{-5} and 10^{-4} M). Previous exposure to lower levels of ACh did not affect the magnitude

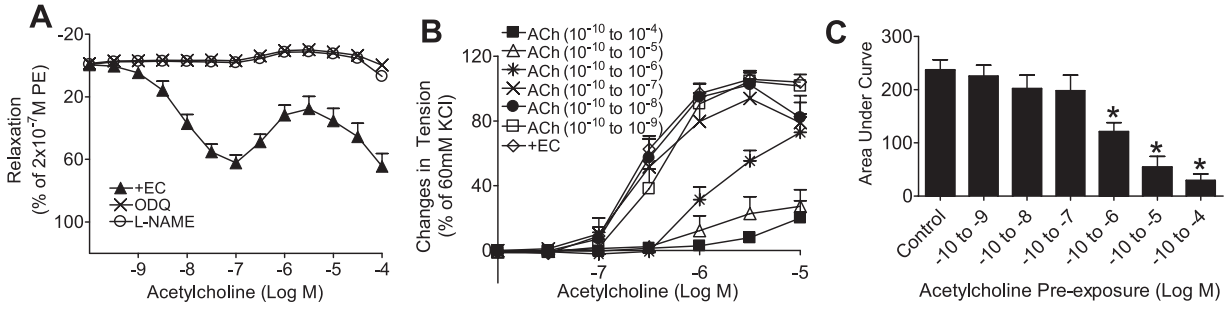

Fig. 4. *A*: relaxations to increasing concentrations of ACh in rings contracted with PE, with or without preincubation of L-NAME or 1*H*-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (ODQ; $n = 11$–13). Endothelium-dependent contractions to ACh in rings with previous exposure to different ranges of ACh are shown with data expressed as a concentration-response curve (*B*) or as area under the curve (*C*); $n = 7$–8. *$P < 0.05$ vs. control (no preexposure to ACh).

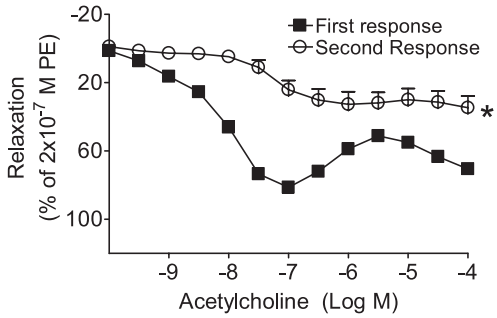

Fig. 5. Sequential concentration-response curves to ACh in the same aortas. Data are expressed as means ± SE; $n = 5$. *$P < 0.05$ vs. first concentration-response curve.

of the subsequent endothelium-dependent contractions (Fig. 4*C*). When a second concentration-response curve was obtained in the same ring, it was shifted significantly to the right compared with the first curve. The maximal relaxation was significantly less. The rebound in tension upon exposure to higher concentrations of the muscarinic agonist was no longer observed (Fig. 5).

Dose-dependent inhibition by SNP. SNP relaxed contracted aortic rings in a concentration-dependent manner. The relaxation was reduced significantly by previous incubation with ODQ. Incubation with L-NAME did not affect the relaxation to SNP (Fig. 6*A*). Previous exposure to progressively increasing concentrations of SNP inhibited the subsequent EDCF-mediated responses in a concentration-dependent manner (Fig. 6*B*). The greatest inhibition was observed when the rings were exposed previously to the highest range of concentrations tested (10^{-10} to 10^{-4} M). Exposure to low concentrations of SNP also inhibited endothelium-dependent contractions, because such inhibition was observed in rings exposed to 10^{-9} M SNP (Fig. 6*C*).

Preexposure to thrombin. Thrombin produced a dose-dependent relaxation in rings contracted with phenylephrine. The maximal relaxation was obtained at 10 U/ml thrombin (Fig. 7*A*). Previous exposure to different thrombin concentrations did not reduce subsequent EDCF-mediated contractions. In fact, previous exposure to 0.001–1 U/ml thrombin significantly augmented the subsequent endothelium-dependent contractions (Fig. 7, *B* and *C*).

Exposure time. Previous exposure to ACh or SNP for 5 min inhibited subsequent EDCF-mediated responses marginally, whereas 20 min of preexposure significantly impaired subsequent endothelium-dependent contractions. When preexposure time was extended to 40 min, a near complete inhibition was observed (Fig. 8).

Role of nitric oxide. Endothelium-dependent contractions to ACh were reduced in rings exposed previously to SNP (10^{-10} to 10^{-4} M) (Fig. 9*A*) or ACh (10^{-10} to 10^{-4} M) (Fig. 9*B*). The inhibitory effect of previous exposure to SNP was significantly but not completely prevented by incubation with ODQ before the initial application of the nitric oxide donor ($P < 0.05$) (Fig. 9*A*). ODQ did not significantly prevent the inhibitory effect of previous exposure to ACh (Fig. 9*B*). L-NAME did not significantly affect the inhibitory effect of previous exposure to either SNP or ACh (Fig. 9). Endothelium-dependent contractions to the calcium ionophore A-23187 were reduced in rings previously exposed to SNP (10^{-10} to 10^{-4} M) (Fig. 9*C*). This inhibitory effect of previous exposure to SNP was not significantly reversed by the incubation of ODQ before the initial application of the nitric oxide donor (Fig. 9*C*).

TP receptor sensitivity. Previous exposure to either ACh or SNP did not significantly affect the concentration-contraction curves to the thromboxane mimetic U-46619 (Fig. 10).

DISCUSSION

The subsequent endothelium-dependent contractions to ACh in SHR aortic rings almost were abolished when the presence of the endothelium was tested at the start of experiments using cumulative concentrations of ACh during contractions to phenylephrine. When the presence of endothelium was not tested, ACh evoked endothelium-dependent contractions similar to those reported previously (1, 17, 36, 38). These contractions require the activation of muscarinic receptors (2), activity of cyclooxygenase-1 (COX1) (11), and the activation of TP receptors (1, 38). The present results with indomethacin and S-18886 confirm the latter two conclusions.

The present study demonstrates that previous exposure to ACh exerts a long-term inhibitory effect on the production and/or actions of EDCF. Previous studies have used low concentration of ACh or ACh with immediate washouts to test the presence of the endothelium before conduction of EDCF studies (13, 20). Because the inhibitory effect of previous exposure to ACh is concentration and time dependent, the magnitude of EDCF-mediated responses in such studies was not, or perhaps only modestly, affected. Other studies of endothelium-dependent contractions used thrombin to test the presence of the endothelium (3, 38). In the present study, the use of thrombin did not reduce the magnitude of subsequent

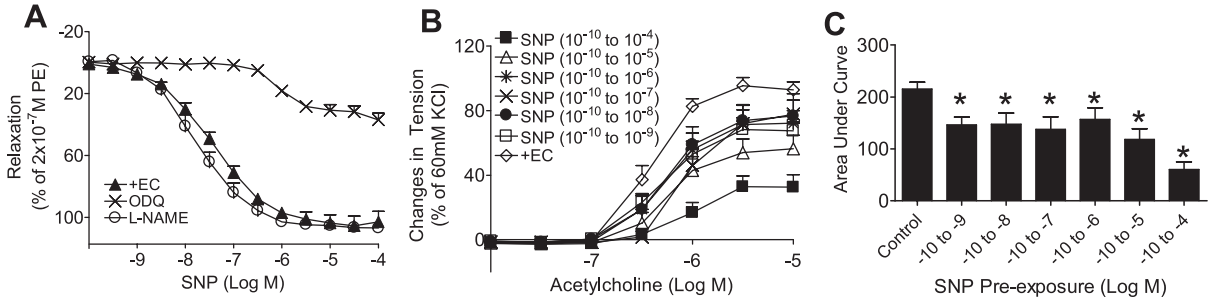

Fig. 6. *A*: relaxations to increasing concentrations of SNP in rings contracted with PE, with or without preincubation of L-NAME or ODQ; $n = 11$–13. Endothelium-dependent contractions to ACh in rings with previous exposure to different ranges of SNP are shown with data expressed as a concentration-response curve (*B*) or as area under the curve (*C*); $n = 7$–8. *$P < 0.05$ vs. control (no preexposure to SNP).

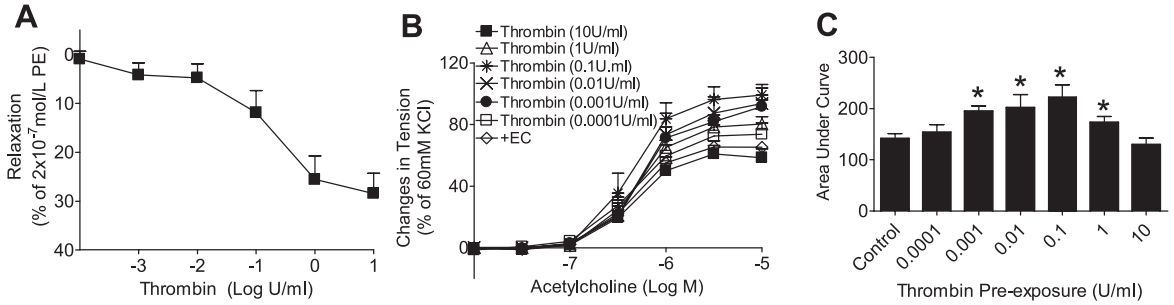

Fig. 7. *A*: relaxations to thrombin in rings contracted with PE; $n = 7$. Endothelium-dependent contractions to ACh in rings with previous exposure to different concentrations of thrombin are shown with data expressed as a concentration-response curve (*B*) or as area under the curve (*C*); $n = 7$–20. *$P < 0.05$ vs. control (no preexposure to thrombin).

EDCF-mediated response, presumably because the enzyme releases minimal amounts of nitric oxide. In fact, preexposure to moderate amounts of thrombin potentiated endothelium-dependent contractions. Thrombin increases the expression of phospholipase A_2, which functions to metabolize membrane phospholipids and contribute to the arachidonic acid pool (8, 26). Thrombin also can increase the expression of COX1 (26). These two actions could explain the augmented endothelium-dependent contractions obtained in rings previously exposed to the enzyme.

Judging from the effect of inhibitors of nitric oxide synthase and soluble guanylyl cyclase, nitric oxide can inhibit EDCF-mediated responses acutely (1, 37). Knowing that there are interactions between nitric oxide and EDCF, we anticipated that perhaps the mediator causing the long-term inhibition of EDCF of ACh preexposure could be nitric oxide. To test this hypothesis, we added SNP cumulatively during contractions to phenylephrine, before the production of EDCF, to see whether a donor of nitric oxide mimics the inhibitory effects of preexposure to ACh. Indeed, previous exposure to high concentrations of SNP reduced the magnitude of the subsequent EDCF-mediated contractions to an extent similar to that caused by preexposure to ACh. At these concentrations, there was sufficient release of nitric oxide to relax the rings maximally. These observations provide evidence that nitric oxide possesses long-term inhibitory effects on the release and/or the action of EDCF.

ACh did not induce full relaxation of the SHR aorta. However, previous exposure to the muscarinic agonist also curtailed subsequent EDCF-mediated responses. Inactivation became apparent when the concentration of ACh was raised to 10^{-6} M or higher. These concentrations are associated with endothelium-dependent contractions in the absence of preexposure to the muscarinic agonist. Previous exposure to a low concentration of ACh did not affect subsequent EDCF-mediated responses. By contrast, even minimally active concentrations (as low as 10^{-9} M) of SNP reduced the magnitude of the subsequent endothelium-dependent contractions. The difference between ACh and SNP could have arisen because the relaxations caused by the latter were longer lasting. Indeed, relaxations to SNP were well preserved during the 40-min observation period, whereas those to ACh were transient.

Previous exposure to ACh inhibits EDCF-mediated responses by a different mechanism than exposure to SNP. This conclusion is based on the observations that incubation with ODQ to prevent the activation of guanylyl cyclase only restored the subsequent EDCF-mediated responses to ACh in rings previous exposed to the nitric oxide donor, not to the muscarinic agonist. This finding suggests that nitric oxide only mediates a minor part of the long-term EDCF inhibition of rings preexposed to ACh. However, nitric oxide appears to be the major mediator of the inhibition caused by SNP. A surprising finding was that ODQ did not significantly prevent the inhibitory effect of SNP on endothelium-dependent contractions evoked by the calcium ionophore A-23187.

Although previous exposure to ACh reduced EDCF-mediated contractions to ACh itself, it did not affect EDCF-mediated contractions to the calcium ionophore A-23187. This finding suggests that ACh exerts its inhibitory role on EDCF at an early step of the signaling cascade, an event that is upstream of the increase in endothelial calcium concentration, because A-23187 bypasses these events. Downregulation of the specific muscarinic receptor subtype involved in the endothelium-dependent contractions may explain this selective effect. Prolonged exposure to ACh can lead to attenuation of receptor-mediated responses (34). This process of receptor desensitization generally involves phosphorylation of the receptor by G protein-coupled receptor kinases and binding of the inhibitory

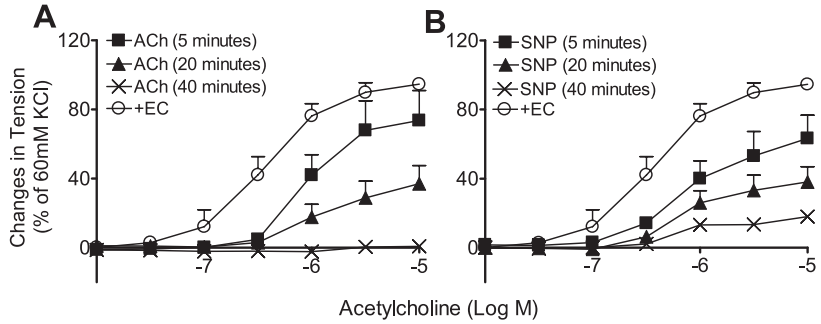

Fig. 8. Contractions to increasing concentrations of ACh in aortic rings preexposed to ACh (10^{-4} M) (*A*) or SNP (10^{-4} M) (*B*) for 5, 20 or 40 min during contractions to PE. Data are expressed as means ± SE; $n = 6$.

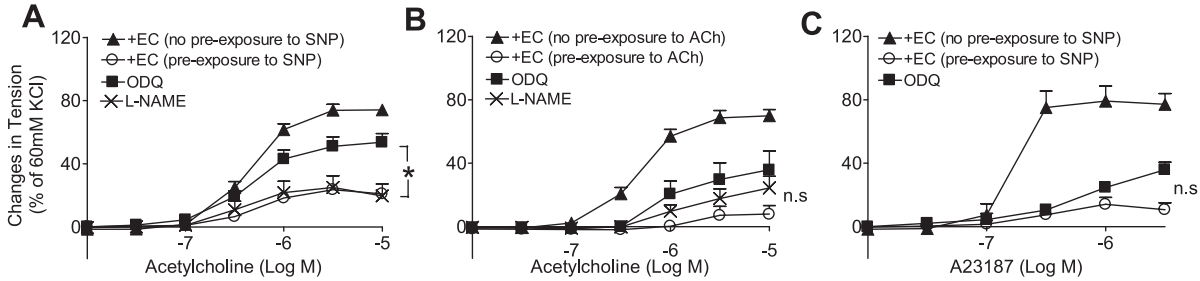

Fig. 9. Contractions to increasing concentrations of ACh in rings with endothelium of rat aorta with or without previous exposure to SNP (n = 13–24) (*A*) or ACh (n = 13) (*B*) in the absence or presence of ODQ or L-NAME before the application of preexposure agent. *$P < 0.05$, ODQ vs. preexposure to SNP; n.s., no significant difference. *C*: contractions to increasing concentrations of the calcium ionophore A-23187 in rings with endothelium of rat aorta with or without previous exposure to SNP in the absence or presence of ODQ before the application of SNP. Data are expressed as means ± SE; n = 4–6.

protein β-arrestin to the phosphorylated receptor, thereby inhibiting further coupling with heterotrimeric G proteins (34). The desensitized phosphorylated receptors can be internalized into the cell interior and undergo intracellular dephosphorylation, after which they recycle back to the plasma membrane as functional receptors or undergo receptor downregulation by targeting the receptors for degradation in the lysosomes (33, 34). Such a process could account for the poor capacity to produce endothelium-dependent contractions after preexposure to a high concentration of ACh for a prolonged time. The observation that the concentration-relaxation curve to ACh is blunted upon repeated exposure supports this hypothesis.

Previous exposure to SNP presumably inhibits EDCF-mediated responses through an event that is downstream of the rise in calcium concentration, because it also inhibited endothelium-dependent contractions to the calcium ionophore A-23187. Changes in sensitivity of the smooth muscle to EDCF or alterations in sensitivity of the contractile machinery are not likely to be the reason for the decreased endothelium-dependent contractions, because the response to U-46619 that activates TP receptors (5) is comparable in all rings whether or not they were previously exposed to the nitric oxide donor. Therefore, the changes exerted by preexposure to SNP most likely involve modifications of expression and/or activity of genes/proteins involved in endothelium-dependent contractions within the endothelial cells. Nitric oxide can modulate the cyclooxygenase pathway (18). Depending on the cell type and experimental conditions used, NO either stimulates (7, 24, 25, 35), inhibits (15, 28), or does not influence (6, 31) prostanoid synthesis. These contradictory results are difficult to explain. One explanation is the heterogeneity in cellular responses. Alternatively, low levels of NO in endothelial cells may stimulate cyclooxygenase, whereas high levels may inhibit the enzyme. Several mechanisms have been proposed to explain how NO modulates cyclooxygenase activity: *1*) direct binding of NO causes conformational changes in the secondary protein structure of the enzyme(s) (25, 35); *2*) NO interacts with the iron-heme center group, which is needed as a cofactor for cyclooxygenase (25, 35); or *3*) NO regulates eicosanoid production at the level of gene transcription (4, 39). The first two possibilities are independent of cGMP (7, 21, 25). This then implies that the action of cGMP released via stimulation of soluble guanylyl cyclase by nitric oxide (or nitric oxide donors) is to reduce the bioavailability of EDCF, because the action of the latter on TP receptors is not likely to be modified, judging from the experiments with U-46619. Alternatively, the activation of soluble guanylyl cyclase in endothelial cells has an autocrine effect to diminish the activity of the prostanoid synthase(s) involved in the formation of EDCF. The present experiments do not permit further speculation on the exact site

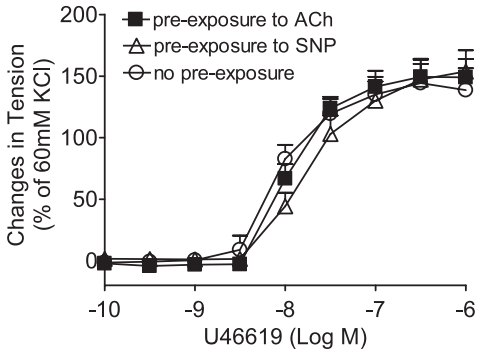

Fig. 10. Contractions to increasing concentrations of U-46619 in rings with endothelium of rat aorta with or without previous exposure to SNP or ACh. Data are expressed as means ± SE; n = 6.

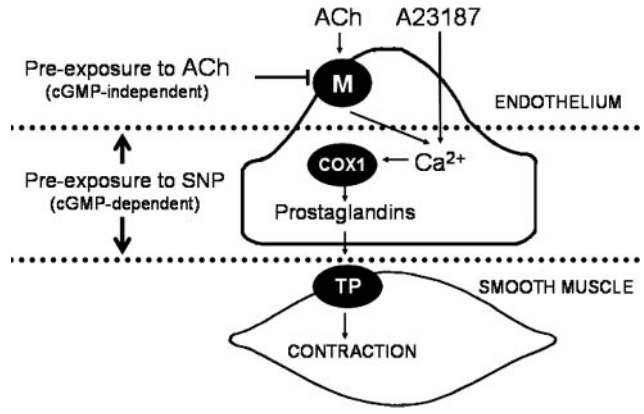

Fig. 11. Proposed sites of action underlying the inhibitory effects of preexposure to ACh and SNP in aortas of spontaneously hypertensive rats (SHR). Preexposure to ACh prevents endothelium-derived contracting factor (EDCF)-mediated responses by interfering with an early step of the signaling cascade that is mainly nitric oxide and cGMP independent, possibly through changes of the muscarinic (M) receptors themselves. Preexposure to SNP mainly suppresses EDCF-mediated responses in a nitric oxide-, cGMP-dependent way. Preexposure to SNP also affected the EDCF-mediated response to the calcium ionophore A-23187, suggesting that cGMP acts at a level downstream of the increase in calcium concentration but nevertheless within the endothelial cells, because the sensitivity of thromboxane-prostanoid (TP) receptors was not altered after preexposure to either ACh or SNP. COX1, cyclooxygenase-1.

at which nitric oxide donors prevent endothelium-dependent contractions in the aorta of SHR.

In summary (Fig. 11), the present experiments suggest that preexposure to ACh prevents EDCF-mediated responses by interfering with an early step of the signaling cascade, which is mainly nitric oxide and cGMP independent, possibly through changes of the muscarinic receptors themselves. Large prior discharge of nitric oxide (as obtained with high concentrations of SNP) can also suppress EDCF-mediated responses in a cGMP-dependent way. Because the responses to the calcium ionophore A-23187 also are affected, cGMP must act at a level downstream of the increase in calcium concentration, which is likely to underlie endothelium-dependent contractions. Changes in sensitivity of the vascular smooth muscle do not seem to be involved. Thus nitric oxide exerts not only an acute but also a long-term inhibitory effect on EDCF-mediated responses.

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