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Evidence that cGMP Is the Mediator of Endothelium-Dependent Inhibition of Contractile Responses of Rat Arteries to α-Adrenoceptor Stimulation

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SUMMARY

Endothelium-derived relaxing factors (EDRFs) have been previously shown to exert an inhibitory influence on the contractile effects of α-adrenoceptor agonists in vascular smooth muscle. α_2-Adrenoceptor agonists such as clonidine have been reported to be particularly susceptible to this effect, and it has been suggested that clonidine acts on α_2 receptors on endothelial cells to stimulate the release of EDRF. EDRF release is known to be accompanied by increased levels of cGMP in many blood vessels, and it is suggested that cGMP exerts an inhibitory influence on the smooth muscle cells, which tends to counteract the contractile effect of the clonidine. This hypothesis was tested in isolated rings of rat aorta and mesenteric artery using the cGMP lowering agent, 6-anilino-5,8-quinolinedione (LY83583). LY83583 markedly decreased resting levels of cGMP in these vascular preparations and completely prevented both the relaxation and the cGMP elevation normally caused by acetylcholine in rat aorta with intact endothelium. These effects of LY83583 are identical to those observed after mechanical disruption of the endothelium. LY83583 also enhanced the contractile responses to norepinephrine and particularly to clonidine in both aorta and mesenteric artery. The effects of LY83583 on contractile responses to both α-adrenoceptor agonists were reversed by low concentrations of 8-bromo-cGMP. Clonidine did not increase cGMP levels in vascular preparations with intact endothelia, in the presence or absence of LY83583. Thus, enhanced release of EDRF by clonidine did not appear to be responsible for the inhibition of its contractile effects observed in the presence of intact endothelial cells. Our results suggest instead that this endothelium-dependent inhibition is due to spontaneous release of EDRF, which results in tonic elevation of cGMP in the vascular smooth muscle. This tonic elevation of cGMP exerts a more marked inhibitory effect against contractions induced by the partial agonist, clonidine, than it does against contractions induced by a full agonist, norepinephrine.

The dependence of some vasodilators, including acetylcholine, on the integrity of the vascular endothelium for their activity is now well established (1). In the presence of an intact endothelium, these agents have been shown to promote the release of a factor, called EDRF, which acts on smooth muscle to produce relaxation. Although the identity of the EDRF and the mechanism by which it produces vasodilation have not been completely determined, there is now substantial evidence implicating cGMP as the mediator of its relaxant effects in vascular smooth muscle (2–6).

Recently, it has become apparent that contractile responses of some arteries to α-adrenoceptor agonists are enhanced by the removal of the endothelium (7–11). In rat aorta, removal of the endothelium increased responses to the α_2-selective agonists, clonidine and BHT-920, to a greater extent than those to the nonselective agonist, norepinephrine (8, 10). In addition, BHT-920 was reported to produce a small increase in cGMP levels in the presence of an intact endothelium in this preparation (10). Therefore, it was suggested that activation of α_2 receptors located on the endothelium of rat aorta leads to increased release of EDRF, elevation of cGMP levels, and inhibition of contractile responses to stimulation of α receptors located on vascular smooth muscle. However, in a subsequent report, both clonidine and methoxamine were demonstrated to elevate cGMP levels to a similar extent in rat aorta with an intact endothelium, whereas the maximum contractile response to clonidine but not that to methoxamine was enhanced by removal of the endothelium (11). This led to the suggestion that cGMP elevation might not be responsible for the endothelium-dependent inhibition of contractile responses to α-adrenoceptor stimulation in this preparation (11). Thus, there are

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ABBREVIATIONS: EDRF, endothelium-derived relaxing factor; BHT-920, 6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo-[4,5,d]-azepine; 8-bromo-cGMP, 8-bromo-cyclic guanosine 3′,5′-monophosphate; LY83583, 6-anilino-5,8-quinolinedione.

conflicting reports concerning the importance of cGMP in this process.

In the present study, the novel cGMP lowering agent, LY83583, was used to further investigate the role of cGMP in the endothelium-dependent inhibition of contractile responses of rat arteries to α-adrenoceptor agonists. Although its mechanism of action is unknown, LY83583 has been reported to reduce resting cGMP levels and to block the elevation of cGMP in response to agonists in a number of preparations, including guinea pig lung, heart, and cerebellum (12), rabbit heart (13, 14), and rabbit aorta (15). In the present investigation, the effects of clonidine and norepinephrine on the contractile tension of rat aorta and mesenteric artery with intact endothelia were determined in the absence and presence of LY83583. In addition, the influence of LY83583 on resting cGMP levels and on cGMP levels in the presence of clonidine and acetylcholine was assessed in rat aorta with intact endothelium.

Materials and Methods

Male Wistar rats weighing 300–350 g were obtained from the Animal Care Center, University of British Columbia. Animals were sacrificed by stunning followed by decapitation. The thoracic aorta and the superior mesenteric artery were removed from each animal and placed in Krebs solution of composition (mM): NaCl (113), KCl (4.7), $CaCl_2$ (2.5), KH_2PO_4 (1.2), $MgSO_4$ (1.2), $NaHCO_3$ (25), and dextrose (11.5). The vessels were carefully cleaned of fat and adventitia, and were cut into 3-mm (mesenteric artery) or 5-mm (aorta) rings. Only two rings of each vessel were used from each animal. The rings were suspended between the bases of two triangular-shaped wires and placed in isolated tissue baths containing Krebs solution at 37°, oxygenated with 95% O_2/5% CO_2. One wire was attached to a fixed tissue support, while the other wire was attached by a silk thread to a Grass FT.03 force displacement transducer. Responses were recorded on a Beckman model 611 dynograph or a Grass model R7 polygraph. Rings of aorta were placed under a resting tension of 2.0 g, whereas rings of mesenteric artery were placed under a resting tension of 1.0 g, and all tissues were allowed to equilibrate for 90–120 min before the experiments were begun. All experiments were done in the presence of 1 μM timolol, 0.1 μM desipramine, and 1 μM hydrocortisone, to eliminate the effects of β receptors, and neuronal and extraneuronal uptake, respectively.

Contractility studies. In a previous investigation in rabbit aorta, 10 μM LY83583 was found to completely block both the relaxant response and the increase in cGMP levels produced by acetylcholine (15). Therefore, the effect of this concentration of LY83583 on contractile responses of aorta and mesenteric artery to norepinephrine and clonidine was determined. One tissue of each pair of aortas and mesenteric artery preparations was incubated with 10 μM LY83583 for 20 min, while the other tissue was treated for the same period with vehicle (0.1% ethanol). A cumulative dose response curve to clonidine was then obtained in each tissue. Tissues were washed for 60 min, reincubated with LY83583 or vehicle, and a cumulative dose response curve to norepinephrine was obtained. Following completion of the norepinephrine curve, all tissues were washed for a further 60 min, and the ability of 1 μM acetylcholine to relax a contraction induced by 0.3 μM norepinephrine was determined.

In some experiments, the effect of 8-bromo-cGMP on contractile responses of aorta and mesenteric artery to clonidine and norepinephrine in the presence of LY83583 was determined. In these experiments, a control dose response curve to either clonidine or norepinephrine was obtained in each preparation. Tissues were washed for 60 min, treated with 10 μM LY83583 for 20 min, and a second dose response curve to the agonist was obtained. Following a second 60-min wash period, tissues were treated with a combination of 10 μM LY83583 and 10 μM 8-bromo-cGMP for 20 min, and a third dose response curve to the agonist was obtained in each preparation.

At the completion of each experiment, tissues were lightly blotted, measured, and weighed. The cross-sectional area of each preparation was calculated using the following formula: cross-sectional area (mm^2) = weight (mg)/length (mm) × density (mg/mm^3). The density of the arteries was assumed to be 1.05 mg/mm^3. The response of each preparation to the agonists was then calculated as the increase in tension (g) in response to each concentration of agonist/cross-sectional area of tissue. pD_2 ($-\log ED_{50}$) values were also calculated for each norepinephrine dose response curve. Responses to clonidine in the absence of LY83583 were too small for reliable calculation of pD_2 values.

Measurement of cGMP and cAMP levels. Rings of aorta were set up and equilibrated in isolated tissue baths as described above. At various times after the addition of drugs, tissues were frozen with clamps cooled in liquid N_2, and then were stored at −80° until assayed for cGMP and cAMP. In some experiments, the endothelium was removed before the tissues were placed in the baths. This was done by placing the rings of aorta on a metal rod and gently rotating them. In preliminary experiments, we found that this was sufficient to abolish the relaxant response to acetylcholine.

cGMP levels were also measured in some mesenteric artery preparations. In order to obtain sufficient tissue to perform the assay, the entire mesenteric vascular bed was removed and cleaned, as much as possible, of fat and mesentery. The preparations were incubated in beakers containing Krebs solution, maintained at 37°, and oxygenated with 95% O_2/5% CO_2 for 2 hr. The tissues were then treated with drug, frozen, and stored as described above.

The frozen aorta and mesenteric artery preparations were homogenized in 1 ml of 6% trichloroacetic acid and centrifuged at 6000 × g for 40 min. The supernatants were extracted four times in 5 volumes of water-saturated ether, and aliquots were taken for cyclic nucleotide assay. cAMP levels were determined in untreated supernatant using radioimmunoassay kits obtained from Becton Dickinson Ltd. (Mississauga, Ontario). Following acetylation, cGMP levels were determined in supernatant using radioimmunoassay kits obtained from New England Nuclear (Lachine, Quebec).

Drugs used. Clonidine hydrochloride, (−)-norepinephrine hydrochloride, and acetylcholine chloride were obtained from Sigma (St. Louis, MO). Stock solutions of clonidine and norepinephrine were prepared fresh daily in distilled water containing 1 mg/ml ascorbic acid. LY83583 was a gift from the Eli Lilly Co. (Indianapolis, IN). LY83583 was dissolved in ethanol such that a final bath concentration of 0.1% ethanol was obtained.

Statistics. Results were compared for significant differences using one-way analysis of variance. When more than two groups of data were compared, results were further analyzed using the Newman-Keuls test. Results were considered to be significantly different at $p < 0.05$.

Results

Contractile responses of rat aorta and mesenteric artery with intact endothelia to clonidine and norepinephrine are shown in Fig. 1. The integrity of the endothelium was confirmed by the presence of a relaxant response to acetylcholine. In the absence of LY83583, clonidine produced almost no detectable increase in tension in either artery, although both vessels responded to norepinephrine with a marked increase in tension. Incubation of aorta with LY83583 resulted in a leftward shift of the norepinephrine dose response curve, with no change in the maximum response to this agonist. The norepinephrine pD_2 value increased significantly, from a control of 7.23 ± 0.22 to 8.21 ± 0.25 (mean ± SE, $n = 5$) in the presence of LY83583. The effects of LY83583 on responses of aorta to this agonist were very similar to those of endothelium removal, which resulted in an increase in the norepinephrine pD_2 value to 8.14 ± 0.25, with no change in the maximum response to this agonist. In the absence of endothelium, the norepinephrine pD_2

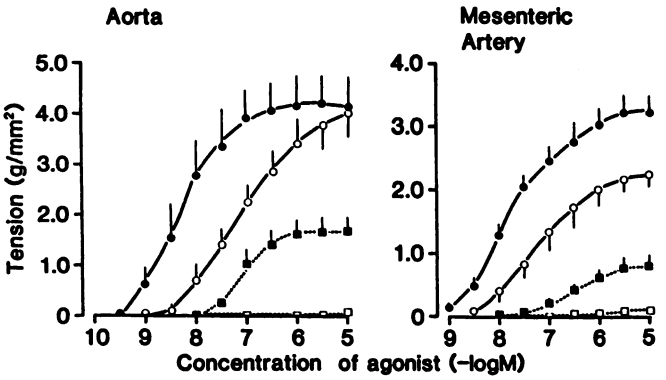

Fig. 1. The influence of LY83583 on cumulative dose response curves to norepinephrine (●, ○) and clonidine (■, □) in rat aorta and mesenteric artery with intact endothelia. ○, □, responses to the agonist in untreated preparations; ●, ■, responses in preparations pretreated for 20 min with 10 μM LY83583. Each curve represents data from five to nine preparations (mean ± standard error).

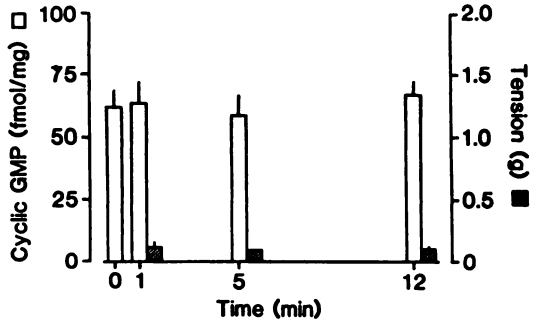

Fig. 2. The effects of 2 μM clonidine on tension and cGMP levels in rat aorta with intact endothelium. Aortic rings were clamp-frozen at various times after addition of clonidine. Tension and cGMP levels were determined in the same preparations (as described in Materials and Methods). Each *bar* represents the mean ± standard error of 6–11 experiments.

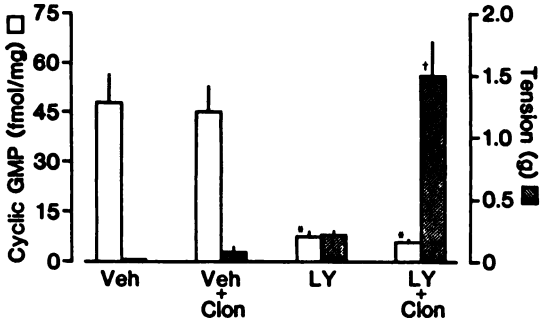

Fig. 3. The effects of clonidine and LY83583, alone and in combination, on tension and cGMP levels in rat aorta with intact endothelium. *Veh*, aortic rings frozen after treatment with 0.1% ethanol for 22 min. *Veh + Clon*, tissues frozen after treatment with 0.1% ethanol for 22 min with 1 μM clonidine added for the final 12 min. *LY*, tissues frozen after treatment with 10 μM LY83583 alone for 22 min. *LY + Clon*, tissues frozen after exposure to 10 μM LY83583 for 22 min with 1 μM clonidine added for the final 12 min. Each *bar* represents the mean ± standard error of five to six experiments. *, $p < 0.05$ compared to *Veh* and *Veh + Clon* groups; †, $p < 0.05$ compared to *all other groups*.

value in the presence of LY83583 was 8.21 ± 0.27, which was not significantly different from that in the absence of LY83583, indicating that this substance had no further effect on responses to norepinephrine in the absence of endothelium. Responses of aorta to clonidine were greatly increased by treatment with LY83583. Maximum responses to clonidine were increased in the presence of LY83583 to approximately 40% of the maximum norepinephrine response. Treatment of mesenteric artery with LY83583 resulted in increases in both the norepinephrine maximum response and pD_2 value. In this set of experiments, the maximum response to norepinephrine was increased by approximately 40% relative to control, while the pD_2 value was significantly increased, from a control of 7.22 ± 0.10 to 7.71 ± 0.07 (mean ± SE, $n = 9$) in the presence of LY83583. As was found in aorta, the effects of LY83583 on responses of mesenteric artery to norepinephrine were very similar to those of removal of the endothelium. The norepinephrine pD_2 value in the absence of endothelium was 7.80 ± 0.10, which was significantly greater than the control pD_2 value, but not significantly different from the pD_2 value in the presence of LY83583. Following removal of the endothelium, LY83583 had no further effect on the norepinephrine response, the pD_2 value for this agonist being 7.69 ± 0.16 under these conditions. Responses of mesenteric artery to clonidine were also markedly enhanced in the presence of LY83583, although not to quite the same extent as in aorta. In these experiments, in the presence of LY83583 the maximum response to clonidine was increased to approximately 25% of the maximum response to norepinephrine.

cGMP levels and tension in rat aorta at various times after the administration of clonidine are shown in Fig. 2. A functional endothelium was assumed to be present in these tissues because the control cGMP levels (62.0 ± 7.7 fmol/mg wet weight of tissue) were much higher than those found after removal of the endothelium (13.1 ± 3.5 fmol/mg wet weight, $n = 6$), and because clonidine produced only a very small increase in tension. However, clonidine had no detectable effect on cGMP levels at any of the time points measured. This is further illustrated in Fig. 3, which shows the effects of clonidine on tension and cGMP levels in the absence and presence of LY83583. LY83583 alone produced a significant reduction in cGMP levels and a small increase in tension. Basal cGMP levels in the presence of LY83583 (8.0 ± 1.6 fmol/mg wet weight) were not significantly different from those found in rat aorta after removal of the endothelium. Clonidine had no effect on cGMP in the presence or absence of LY83583, but, as expected, it produced a much greater increase in tension in the presence of LY83583.

Neither clonidine nor LY83583 had any effect on cAMP levels in these experiments. For example, cAMP levels in aortic rings were 261 ± 17 fmol/mg wet weight of tissue in vehicle-treated controls, 239 ± 36 fmol/mg in clonidine-treated controls, 230 ± 27 fmol/mg in LY83583-treated preparations, and 228 ± 20 fmol/mg in preparations treated with both LY83583 and clonidine (means ± SE, $n = 5–6$).

The effects of clonidine and LY83583 on cGMP levels in mesenteric artery preparations were qualitatively similar to those found in aorta (Table 1). The large standard error associated with control cGMP levels in mesenteric artery preparations may have been due to variations in the integrity of the endothelium in these preparations, although this could not be verified under the conditions of these experiments. As was the case in the aorta, LY83583 treatment resulted in a large decrease in cGMP levels compared to control. Clonidine had no significant effect on cGMP levels in the presence or absence of LY83583.

The influence of LY83583 on responses of rat aorta with

TABLE 1

Effects of clonidine and LY83583 on cGMP levels in rat mesenteric artery

Clonidine-treated preparations were exposed to 1 μM clonidine for 12 min, LY83583-treated preparations were exposed to 10 μM LY83583 for 22 min, and LY83583 + clonidine-treated preparations were exposed to 10 μM LY83583 for 22 min with 1 μM clonidine added for the last 12 min.

Treatment	N	cGMP
		fmol/mg tissue
Control	6	12.8 ± 6.1
Clonidine	6	12.4 ± 3.5
LY83583	6	2.6 ± 0.3
LY83583 + clonidine	6	4.2 ± 0.9

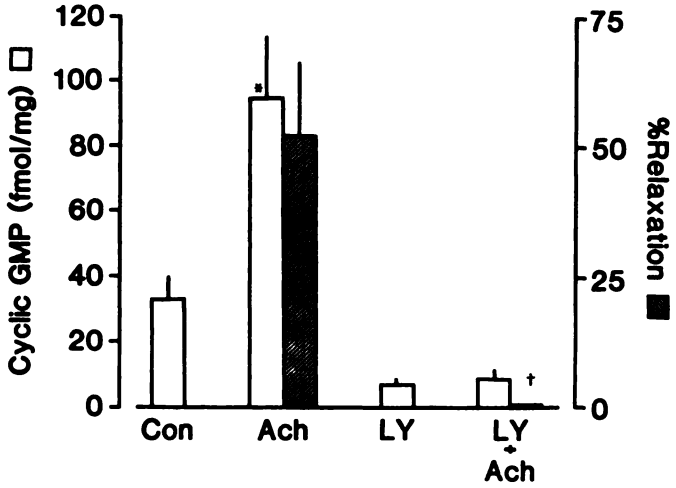

Fig. 4. The effects of LY83583 on acetylcholine-induced relaxation and cGMP levels in rat aorta with intact endothelium. Effects of acetylcholine on tension are expressed as per cent relaxation of norepinephrine-induced contractions. *Con*, tissues frozen after treatment with 0.3 μM norepinephrine for 12 min. *Ach*, tissues frozen after treatment with 0.3 μM norepinephrine for 12 min with 1 μM acetylcholine added for the final 2 min. *LY*, tissues frozen after treatment with 10 μM LY83583 for 22 min. *LY + Ach*, tissues frozen after treatment with 10 μM LY83583 for 22 min with 0.3 μM norepinephrine added for the last 12 min and 1 μM acetylcholine added for the final 2 min. Each *bar* represents the mean ± standard error of five to six experiments. *, $p < 0.05$ compared to all other groups; †, $p < 0.05$ compared to the *Ach* group.

intact endothelium to acetylcholine is shown in Fig. 4. Acetylcholine alone produced a significant rise in cGMP levels and a 50% relaxation of the contraction to 0.3 μM norepinephrine. LY83583 reduced basal cGMP levels and completely abolished both the increase in cGMP and the relaxant response of aorta to acetylcholine.

In order to determine whether cGMP could reverse the effects of LY83583 on contractile responses of rat arteries to α-adrenoceptor stimulation, the effects of a low concentration of 8-bromo-cGMP on responses of aorta and mesenteric arteries to clonidine and norepinephrine in the presence of LY83583 were measured. The effects of LY83583 alone on contractile responses to norepinephrine and clonidine were similar to those observed in our earlier experiments. In aorta, LY83583 increased the norepinephrine pD_2 value from a control of 7.23 ± 0.04 to 7.78 ± 0.03 (mean ± SE, $n = 4$) and enhanced the maximum response to clonidine (Fig. 5). Addition of 10 μM 8-bromo-cGMP in the presence of LY83583 shifted the norepinephrine response to the right, decreasing the pD_2 value to 6.62 ± 0.12, without affecting the maximum response to this agonist. However, it depressed the maximum clonidine response to a level slightly less than that found in the absence of LY83583.

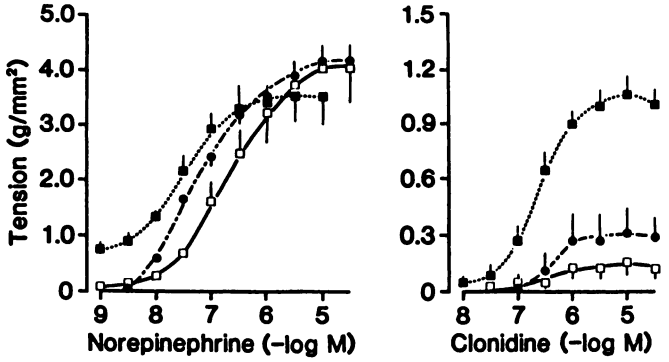

Fig. 5. Reversal by 8-bromo-cGMP of effects of LY83583 on cumulative dose response curves to norepinephrine and clonidine in rat aorta with intact endothelium. Dose response curves to the agonists were first obtained in untreated preparations (●), then in the presence of 10 μM LY83583 (■), and finally in the presence of 10 μM LY83583 plus 10 μM 8-bromo-cGMP (□) as described in Materials and Methods. Each curve represents data from four preparations (mean ± standard error).

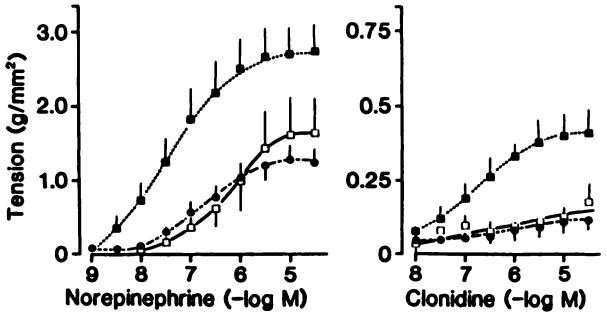

Fig. 6. Reversal by 8-bromo-cGMP of effects of LY83583 on cumulative dose response curves to norepinephrine and clonidine in rat mesenteric artery with intact endothelium. Dose response curves to the agonists were first obtained in untreated preparations (●), then in the presence of 10 μM LY83583 (■), and finally in the presence of 10 μM LY83583 plus 10 μM 8-bromo-cGMP (□) as described in Materials and Methods. Each curve represents data from four preparations (mean ± standard error).

In mesenteric artery, LY83583 treatment enhanced maximum responses to both norepinephrine and clonidine, and increased the norepinephrine pD_2 value from a control of 6.80 ± 0.14 to 7.34 ± 0.22 (mean ± SE, $n = 4$). As in aorta, these effects were completely reversed by 8-bromo-cGMP treatment (Fig. 6).

Discussion

In the present investigation, the cGMP lowering agent, LY83583, was found to enhance the contractile responses of rat aorta and mesenteric artery to the α-adrenoceptor agonists, clonidine and norepinephrine, and to block the relaxant responses of these vessels to acetylcholine. The effects of LY83583 on responses to these three agonists are similar to those of removing the endothelium from rat aorta and mesenteric artery (8–11, 16), and LY83583 had no further effect on responses of these arteries to norepinephrine and clonidine in the absence of endothelium. These results suggest that LY83583 is interfering with the ability of the endothelium to modulate responses of rat arteries to α-adrenoceptor stimulants, as well as to muscarinic agonists.

LY83583 alone caused a small and variable increase in tension, and a marked reduction in cGMP levels in rat aorta and mesenteric artery with intact endothelia. In rat aorta, cGMP levels in the presence of LY83583 were similar to those observed

after removal of the endothelium. A reduction in cGMP levels on removal of the endothelium and on exposure to the guanylate cyclase inhibitor, methylene blue, has previously been reported to occur in rat aorta (10), and it has been proposed that in this tissue, as well as in rabbit aorta, EDRF is released spontaneously (10, 17, 18). The results of the present investigation suggest that EDRF is released continuously in rat mesenteric artery as well as in aorta, and that in both preparations, EDRF may exert a mild relaxant effect through tonic elevation of cGMP levels. Elimination by LY83583 of this tonic inhibitory influence is presumably responsible for the increase in tension observed after LY83583 treatment.

In rat aorta with intact endothelium, acetylcholine relaxed norepinephine-induced contractions and significantly elevated cGMP levels, as had been reported previously (5). Both the relaxant response and the increase in cGMP produced by acetylcholine were blocked by pretreatment with LY83583. These data are consistent with the hypothesis that acetylcholine increased EDRF release in rat aorta, and that cGMP is the mediator of the relaxant effects of this agonist in the presence of an intact endothelium. In contrast, clonidine had no detectable effect on cGMP levels in rat aorta or mesenteric artery with intact endothelia in either the absence or presence of LY83583. This suggests that clonidine does not stimulate EDRF release in these arteries. However, when basal cGMP levels were reduced by LY83583, the contractile effects of clonidine and, to a lesser extent, norepinephrine were enhanced in both blood vessels. This suggests that the endogenous cGMP levels resulting from spontaneous EDRF release are sufficient to inhibit responses to α-adrenoceptor agonists in these arteries. Further evidence consistent with a role for cGMP in this process is provided by our experiments with 8-bromo-cGMP. In these experiments, administration of a low concentration of 8-bromo-cGMP reversed the enhancement by LY83583 of contractile responses to α-adrenoceptor agonists in both rat aorta and mesenteric artery. Thus, decreasing endogenous cGMP levels with LY83583 enhanced the contractile effects of α-adrenoceptor agonists in these tissues, whereas addition of exogenous cGMP to these cGMP-depleted tissues returned the responses to control levels. It is possible that other physiological antagonists might also reverse the effects of LY83583 on contractile responses to α-adrenoceptor agonists. Nevertheless, these results demonstrate that changes in cGMP levels could account for the observed effects.

α_2-Adrenoceptors were originally proposed to be present on the endothelium of rat aorta and to cause release of EDRF, because responses to the α_2-selective agonists, clonidine and BHT-920, were enhanced to a much greater extent by removal of the endothelium than were those to the nonselective agonist, norepinephrine, or to the α_1-selective agonist, methoxamine (8, 10). However, it has since been reported that there is no correlation between the magnitude of increase in response to an agonist after removal of the endothelium from rat aorta and the selectivity of the agonist for α_1 or α_2 receptors (9). In addition, it has not been possible to demonstrate the presence of either α_1 or α_2 receptors on the vascular endothelium of rat aorta (9, 19). In the present investigation, responses to clonidine were enhanced to a greater extent than those to norepinephrine in rat aorta and mesenteric artery in the presence of LY83583, although the clonidine response never attained the same magnitude as the norepinephrine response. However, as described above, our results suggest that clonidine does not increase EDRF release in either preparation. An alternative explanation for the greater enhancement of the clonidine response than the norepinephrine response by LY83583 is that clonidine, as a partial agonist, is more susceptible than norepinephrine, a full agonist, to the inhibitory effects of the endogenous cGMP produced by spontaneous EDRF release. This explanation is supported by our results with 8-bromo-cGMP. In rat aorta, in the presence of LY83583, a concentration of 8-bromo-cGMP which shifted the norepinephrine dose response curve to the right, without affecting the maximum response to this agonist, almost abolished the contractile response to clonidine. In mesenteric artery, where maximum responses to norepinephrine as well as to clonidine were enhanced by LY83583, responses to both agonists were returned to control levels by 8-bromo-cGMP. In addition, in preliminary experiments we found that responses to the α_1-adrenoceptor agonist methoxamine, which behaves as a partial agonist relative to norepinephrine in rat aorta and mesenteric artery, were also enhanced to a greater extent than responses to norepinephrine by treatment of tissues with LY83583. In the presence of an intact endothelium, the maximum contractile response of rat aorta to methoxamine was approximately 75% of the maximum norepinephrine response. Treatment of tissues with LY83583 resulted in a leftward shift of the methoxamine dose response curve, and an increase in the maximum methoxamine response to 96% of the maximum norepinephrine response. The maximum response of rat mesenteric arteries with intact endothelia to methoxamine was 55% of the maximum norepinephrine response. Treatment of tissues with LY83583 resulted in a leftward shift in the methoxamine dose response curve and an increase in the maximum methoxamine response of 80% relative to control, although the norepinephrine maximum was increased by only 40%. The results of these two sets of experiments suggest that the degree of enhancement of contractile responses to agonists by LY83583 appears to reflect the relative efficacy of the agonists used, rather than differences in their ability to increase EDRF release or in their selectivity for α-adrenoceptor subtypes. Clonidine has been suggested to act as a partial agonist at α_1-adrenoceptors in rat aorta (20, 21) and it is possible that it is acting in this manner in the present experiments.

The conclusions of the present investigation are in agreement with those of a report from Furchgott's laboratory (22), published during the preparation of this manuscript. These authors found that responses of rat aorta to clonidine were enhanced to a greater extent than those to phenylephrine by the guanylate cyclase inhibitor, hemoglobin, although neither of the α-adrenoceptor agonists increased cGMP levels. When a large proportion of the α-adrenoceptors were eliminated by the irreversible agonist, dibenamine, the response of aorta to phenylephrine in the presence and absence of endothelium resembled the clonidine response in the absence of dibenamine. Therefore, Martin *et al.* (22) concluded that α-adrenoceptor agonists do not stimulate EDRF release in rat aorta, and that the greater susceptibility of the clonidine than the phenylephrine response to the depressant effects of spontaneously released EDRF is due to the lower efficacy of clonidine relative to phenylephrine.

The results of the present investigation, like those of Martin *et al.* (22), do not agree with those of Miller *et al.* (10) and Bigaud *et al.* (11), who reported that α-adrenoceptor agonists

increased cGMP levels in rat aorta with intact endothelium. The reason for the different results obtained in these studies is not clear. In both the present investigation and that of Martin *et al.* (22), cGMP levels were measured in rat aorta which had been suspended under tension in isolated tissue baths, and in which contractile tension was also measured. In the earlier studies, cGMP levels were measured in tissues that were not placed under tension (10, 11). However, in the present investigation, cGMP levels were also measured in preparations of mesenteric artery which were not under tension, and these tissues gave results that were qualitatively similar to those obtained in aorta.

In summary, the results of the present investigation confirm previous reports that contractile responses of rat aorta and mesenteric artery to α-adrenoceptor stimulants are inhibited in the presence of an intact endothelium. However, this inhibition appears to be due to the spontaneous release of EDRF, which results in tonic elevation of cGMP levels, rather than to stimulation of the release of EDRF by the α agonists.

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