In Vivo and *In Vitro* Studies of a Putative Inhibitor of Cyclic Guanosine 3′,5′-Monophosphate Production (43159)

MARILYN A. BRANDT AND KIRK P. CONRAD[1]
Department of Physiology, Dartmouth Medical School, Hanover, New Hampshire 03756

Abstract. **Our main objective was to test the efficacy of 6-anilino-5,8-quinolinedione (LY83583) *in vivo*, a putative inhibitor of cyclic guanosine 3′,5′-monophosphate (cGMP) production. If the drug proved capable of lowering plasma, vascular, and kidney levels of cGMP and of inhibiting the hypotensive effect of sodium nitroprusside and methacholine, then LY83583 could be of potential use in exploring the contribution of cGMP to cardiovascular and renal physiology. We found that when administered to trained conscious rats, LY83583 (1-mg/kg bolus, followed by a 2-hr infusion of 3 mg/kg·hr) decreased plasma cGMP concentration by 36% ($P < 0.01$). Doubling the dosage of drug (2-mg/kg bolus, 6 mg/kg·hr) decreased plasma cGMP by 46% ($P < 0.05$). We next measured tissue levels of cGMP *ex vivo* from rats that had received LY83583 or vehicle for 2 hr. The cGMP content of aortic segments when LY83583 was infused at the low dose, or renal cortical tissue when LY83583 was infused at both doses, was not significantly different from the cGMP content of tissue from rats that had received vehicle. LY83583 in doses up to 10-mg/kg bolus, followed by 6 mg/kg·hr infusion also failed to attenuate the hypotensive response to sodium nitroprusside or methacholine in conscious rats. Last, we tested whether, in our hands, LY83583 could reduce cGMP of aortic segments and kidney cortical slices *in vitro*. We found that after 10 min of incubation, 10^{-5} *M* LY83583 decreased intracellular cGMP by approximately 65% and 50% in aortic and kidney tissues, respectively. In order to ascertain whether LY83583 lowered cGMP by stimulating phosphodiesterase, we incubated tissues with 10^{-4} *M* 3-isobutyl-1-methylxanthine to inhibit the enzyme. In the presence of 3-isobutyl-1-methylxanthine LY83583 still exerted an inhibitory effect on cGMP production by aortic and kidney tissues. In conclusion, although LY83583 is a useful agent to lower renal and vascular tissues levels of cGMP *in vitro*, its efficacy *in vivo* seems doubtful.**

[P.S.E.B.M. 1991, Vol 196]

Schmidt *et al.* (1) reported that 6-anilino-5,8-quinolinedione (LY83583) modulated intracellular levels of cyclic guanosine 3′,5′-monophosphate (cGMP), but not of cyclic adenosine 3′,5′-monophosphate. Specifically, they found that LY83583 attenuated cGMP production in lung, heart, and cerebellar tissue *in vitro*. The mechanism(s) by which LY83583 reduced cGMP was not resolved, but direct inhibition of guanylate cyclase seemed unlikely. In the present study, we both confirmed and extended the observations initially made by Schmidt *et al.* (1).

Our main purpose was to ascertain the efficacy of LY83583 *in vivo*. If the drug proved capable of lowering plasma, aortic, and kidney levels of cGMP and of inhibiting the hypotensive effect of sodium nitroprusside and methacholine, then LY83583 could be used to explore the contribution of cGMP to cardiovascular and renal functions. First, we assessed the efficacy of LY83583 *in vivo*. Plasma levels of cGMP were measured before and after acute administration of the drug or vehicle, and the content of cGMP was determined in aortic and renal tissues obtained from animals in which LY83583 or vehicle had been administered. We also assessed in conscious rats whether LY83583 would inhibit the hypotensive action of sodium nitroprusside and methacholine, compounds which stimulate vascular cGMP synthesis, and thereby produce vasodilation (2). Finally, we tested whether LY83583 could lower

[1] To whom requests for reprints should be addressed at Department of Physiology, University of New Mexico School of Medicine, 915 Stanford N.E., Albuquerque, NM 87131.

Received April 3, 1990. [P.S.E.B.M. 1991, Vol 196]
Accepted July 12, 1990.

0037-9727/91/1961-0030$2.00/0

cGMP concentrations in aortic and kidney tissue *in vitro*.

Materials and Methods

Drugs and Chemicals. 6-Anilino-5,8-quinolinedione (LY83583) was generously supplied by the Lilly Co. (Indianapolis, IN). Cyclic guanosine 3′,5′-monophosphate, Hepes, 3-isobutyl-1-methylxanthine (IBMX), trichloroacetic acid (TCA), bovine serum albumin, γ-globulin, polyethylene glycol, and sodium acetate were obtained from Sigma Chemical Co. (St. Louis, MO). The remaining chemicals were purchased from the following vendors: sterile dimethyl sulfoxide (DMSO) (Research Industries, Salt Lake City, UT), Earle's balanced salt solution (Hazleton, Denver, PA), Ringer's solution (Abbott Laboratories, Abbott Park, IL), and [^{3}H]cGMP and ^{125}I-cGMP (New England Nuclear, Boston, MA).

Plasma Level of cGMP after Administration of LY83583. Female Long Evans rats (Charles River, Wilmington, MA) of approximately 100 days old were accustomed to experimental cages for 3 to 4 hr daily for 1 week. They were then anesthetized with a combination of ketamine (60 mg/kg) and pentobarbital (20 mg/kg) so that catheters could be implanted aseptically into the abdominal aorta and inferior vena cava via the left femoral artery and vein, respectively. The catheters were tunneled subcutaneously under the skin and exteriorized between the scapulae. They were filled with a 1:1 solution of 50% dextrose and 1000 IU of sodium heparin and then plugged with a small stainless steel pin. All animals were allowed at least 7 days of recovery before experimentation began. On the day of the experiment, rats were placed in the experimental cages and infused intravenously with Ringer's solution at 2 to 3 μl/min. After 1 hr, 600 μl of blood were collected in cooled EDTA tubes and centrifuged immediately, and the plasma was stored at −40°C until analyzed for cGMP. Then, a 1-mg/kg bolus of LY83583 in 40 μl of 75% DMSO, or 40 μl of 75% DMSO (vehicle), was administered followed by an infusion of 3 mg/kg·hr LY83583 in 75% DMSO at 2 to 3 μl/min, or vehicle at the same rate. After 2 hr, another plasma sample was obtained and stored as described previously.

cGMP Levels in Kidney and Aortic Tissue after Vehicle or LY83583 Administration *In Vivo*. We tested whether infusion of LY83583 could lower levels of cGMP in tissues subsequently removed from the animal. In the first series of experiments, we used the same protocol of catheter placement and experimental design as described in the previous section. After 1 hr of infusion of Ringer's solution, a blood sample was obtained to establish plasma concentrations of cGMP. Next, vehicle (75% DMSO) or LY83583 (2-mg/kg bolus; 6 mg/kg·hr infusion) was administered to the rat for 2 hr, after which another blood sample was obtained. The rats were anesthetized with an intravenous injection of pentobarbital (60 mg/kg) so that the kidneys could be rapidly excised. Two cortical slices were made and immediately homogenized in cold 5% TCA containing [^{3}H]cGMP as tracer. No more than 5 min elapsed from stoppage of LY83583 infusion to homogenization of the kidney tissue. Another group of rats received LY83583 (1-mg/kg bolus, 3 mg/kg·hr infusion) or vehicle for 2 hr. They were then killed so that the aorta could be removed. Approximately 25 min elapsed—the time it took to process the tissues—before the aortic segments could be quenched in TCA.

We performed a second series of experiments in which the kidney was rapidly excised and frozen in liquid nitrogen, since cGMP metabolism is rapid and the effect of LY83583 may be short-lived once it is washed from tissues. Rats were anesthetized with Inactin (100 mg/kg) supplemented with pentobarbital, as necessary, and maintained at 38°C by a heating pad. A tracheostomy was performed and catheters were placed as described above. Ringer's solution was infused intraarterially at 22 μl/min for the entire experiment. Ringer's solution or LY83583 (2-mg/kg bolus; 6 mg/kg·hr) in 75% DMSO was administered intravenously for 2 hr at 2 to 3 μl/min. At the end of the infusion period, a midline abdominal incision was made so that the left kidney could be rapidly excised and immediately dropped in liquid nitrogen. Chunks of cortex were fractured off while the kidney was immersed in liquid nitrogen. The chunks were then dropped in 2 ml of 5% TCA containing a trace amount of [^{3}H]cGMP and homogenized.

Effect of LY83583 on the Hypotensive Response to Sodium Nitroprusside and Methacholine. Rats were chronically instrumented as described above. For each experiment, animals resided in the experimental cage for 1 hr, after which mean arterial pressure was recorded. Methacholine (0.5 and 4 μg/kg), sodium nitroprusside (2.5 and 10 μg/kg), and isoproterenol (0.2 μg/kg) were administered in random order as intravenous boluses. The volume of infusate was 200 μl and at least 5 min of recovery was allowed between boluses. Each dosage was given in duplicate and the decrement in mean arterial pressure was recorded and averaged. Next, 400 μl of 50% DMSO in Ringer's solution was given over 10 min followed by an infusion of 5.24 μl/min. Beginning 10 min later, mean arterial pressure was recorded and the vasodilatory agents were again administered. Following at least 48 hr of rest, the studies were repeated in the same animals, except that LY83583, dissolved in 50% DMSO, was infused (4-mg/kg or 10-mg/kg bolus, followed by a 6 mg/kg·hr infusion).

***In Vitro* Incubation of Tissues.** We examined whether LY83583 was capable of lowering tissue levels of cGMP *in vitro*. Production of cGMP was assessed in aortic segments and kidney cortical slices obtained from

female Long Evans rats approximately 100 days old. Rats were killed, and the aorta (from the arch to the iliac bifurcation) and kidneys were rapidly removed and placed in incubation media. The incubation media consisted of Earle's balanced salt solution which contained 20 m*M* Hepes (pH 7.4), preequilibrated for 30 min with 95% O_2-5% CO_2. The aorta was dissected of adherent fat and connective tissue, cut into six segments of approximately 0.8 cm length, blotted, and then weighed to the nearest 0.1 mg. Slices of kidney cortex were made with a Staddie-Riggs microtome. Incubations were carried out in sealed vials gassed with 95% O_2-5% CO_2 at 37°C containing either LY83583 (10^{-5} *M* final concentration) or vehicle (an equal volume of 0.9% saline). At specific times (5, 10, and 20 min), tissues were transferred to tubes containing cold 5% TCA with [^{3}H]cGMP as tracer and homogenized. Homogenates were transferred to test tubes and centrifuged (4°C) at 3750*g* for 20 min. The supernatant was processed as described below, so that cGMP could later be assessed by specific radioimmunoassay. The protein pellet was frozen at −40°C for subsequent protein analysis (3).

If LY83583 lowered cGMP levels by stimulating phosphodiesterase, then the addition of a phosphodiesterase inhibitor should mitigate the action of LY83583. To test this hypothesis, aortic segments and kidney cortical slices were incubated as described previously except that the phosphodiesterase inhibitor IBMX (10^{-4} *M* final concentration) was added to all incubation vials.

Processing of Plasma and Tissue. The plasma samples were processed as follows: 250 μl of plasma, [^{3}H]cGMP (1500 cpm; to assess procedural losses) and 250 μl of 10% TCA were vortexed together. The samples were centrifuged, and the supernatant was placed in extraction tubes with two volumes of water-saturated ether. The extraction tubes were agitated for 5 min, after which the upper layer of ether was removed and replaced with fresh ether. This procedure was repeated four more times before placing the tubes in a dry bead bath at 70°C for 45 min. Finally, the volume was restored back to 500 μl with distilled water, and all samples were frozen at −40°C until cGMP analysis.

The same procedure was used to process tissue samples except tissue was placed in 2 ml of TCA with [^{3}H]cGMP. The samples were homogenized and centrifuged, and the supernatant was extracted with ether and processed as described previously. The final volume was restored to 2 ml with distilled water. Again, all samples were frozen at −40°C until cGMP and protein analysis.

Radioimmunoassay of cGMP. cGMP was measured by specific radioimmunoassay. Antibody against cGMP was obtained from Kew Scientific (Columbus, OH) and ^{125}I-cGMP was obtained from Amersham (Arlington Heights, IL) or New England Nuclear (Boston, MA). A standard curve was made over a range of 1–250 fmol of cGMP/tube. Samples were assayed in triplicate (three different final dilutions). Trimethylamine and acetic anhydride (1:2, v/v) were added to all tubes, a procedure that acetylated cGMP thereby increasing the sensitivity of the assay (4). Further details of the assay and the validation of it in our laboratory have been described previously (5).

Statistical Analysis. Paired *t* tests were applied when appropriate. Analysis of variance was performed followed by an appropriate post hoc test if overall significance was reached. A value of $P < 0.05$ was considered to be significant.

Results

Effect of *In Vivo* Administration of LY83583 on Plasma cGMP. Infusion of Ringer's or 75% DMSO (vehicle) did not significantly alter plasma cGMP levels (Table I). However, administration of LY83583 (1-mg/kg bolus, 3 mg/kg·hr) lowered plasma cGMP by 36% ($P < 0.01$, $n = 8$ rats). Infusion LY83583 at a higher dose (2-mg/kg bolus, 6 mg/kg·hr) lowered plasma cGMP by 46% ($P < 0.05$, $n = 4$ rats).

cGMP Levels in Kidney and Aortic Tissue after Vehicle or LY83583 Administration *In Vivo*. Following the infusion of 75% DMSO (vehicle) or LY83583 (2-mg/kg bolus; 6 mg/kg·hr) in 75% DMSO to conscious rats for 2 hr, renal cortical levels of cGMP were 371.9 ± 30.8 fmol/mg protein ($n = 4$ rats) and 382.6 ± 24.8 fmol/mg protein ($n = 4$ rats), respectively ($P > 0.05$). In aortic tissues, cGMP levels were 1000.7 ± 81.2 fmol/mg protein (samples in duplicate, $n = 4$ rats) following vehicle infusion and 1189.0 ± 57.4 fmol/mg protein (samples in duplicate, $n = 4$ rats) following LY83583 infusion ($P > 0.05$). Administration of Ringer's solution alone (24 μl/min) or Ringer's solution (22 μl/min) plus LY83583 in 75% DMSO (2–3 μl/min) to anesthetized rats for 2 hr followed by immediate immersion of the kidney in liquid nitrogen also failed to reveal differences

Table I. Plasma cGMP before and after LY83583 Infusion[a]

	Control (pmol/ml)	Experimental (pmol/ml)
Ringer's ($n = 8$)	11.2 ± 1.4	11.9 ± 2.7
DMSO ($n = 18$)	10.1 ± 0.9	9.5 ± 1.0
LY83583 ($n = 8$) (low dose)	7.3 ± 1.2	4.7 ± 1.3[b]
LY83583 ($n = 4$) (high dose)	13.3 ± 1.1	7.3 ± 0.4[b]

[a] Values are mean ± SE. Plasma cGMP was assessed in conscious rats. The control value was obtained 1 hr after infusion of Ringer's solution and the experimental value after 2-hr administration of Ringer's plus 75% DMSO or LY83583 (low dose, 1-mg/kg bolus, 3 mg/kg·hr; high dose, 2-mg/kg bolus, 6 mg/kg·hr) in 75% DMSO. Note that control and experimental levels were assessed in the same rat.
[b] $P < 0.05$ control versus experimental by paired *t* test.

Table II. Effect of LY83583 on Depressor Responses to Methacholine, Sodium Nitroprusside, and Isoproterenol in Conscious Rats[a]

	Methacholine		Sodium nitroprusside		Isoproterenol (0.2 μg/kg)
	0.5 μg/kg	4.0 μg/kg	2.5 μg/kg	10 μg/kg	
Control[b]	12 ± 1	26 ± 1	13 ± 1	22 ± 2	18 ± 1
DMSO	12 ± 1	22 ± 2	8 ± 1[c]	15 ± 1	12 ± 2[c]
Control	10 ± 1	24 ± 1	12 ± 1	19 ± 1	14 ± 2
LY83583 (4 mg/kg, 6 mg/kg·hr)	10 ± 0	21 ± 1	8 ± 1	16 ± 2	11 ± 1
Control[d]	12 ± 1	26 ± 1	12 ± 1	21 ± 1	16 ± 2
DMSO	13 ± 1	24 ± 3	8 ± 1	15 ± 2[c]	13 ± 3
Control	11 ± 1	28 ± 1	11 ± 1	22 ± 2	19 ± 3
LY83583 (10 mg/kg, 6 mg/kg·hr)	10 ± 1	26 ± 3	7 ± 1[c]	14 ± 1[c]	10 ± 1

[a] These values (mean ± SE) are mm Hg decreases in arterial pressure.
[b] The vasodilators were administered as boluses to four chronically instrumented rats before (control) and after administration of the vehicle for LY83583 (400-μl bolus of 50% DMSO over 10 min followed by an infusion of 5.24 μl/min). After at least 48 hr of rest, the rats were again studied in the same manner except that LY83583 was administered (4-mg/kg bolus over 10 min, followed by an infusion of 6 mg/kg·hr).
[c] $P < 0.05$ versus control.
[d] The same protocol as described in footnote *b* was performed, except that a larger dose of LY83583 was administered, 10 mg/kg over 10 min, followed by an infusion of 6 mg/kg·hr. (See Materials and Methods for details.)

Table III. Time Course of cGMP Production *In Vitro* with and without LY83583[a]

	fmol/mg protein		
	5 min	10 min	20 min
Aortic segments			
Control	577 ± 210	922 ± 474	642 ± 114
LY83583	387 ± 21	322 ± 45	370 ± 50
Kidney cortical slices			
Control	150 ± 26	148 ± 37	129 ± 24
LY83583	119 ± 37	74 ± 13	81 ± 25

[a] Values are mean ± SE. Aortic segments and kidney cortical slices were incubated either with or without LY83583 (10^{-5} *M*) for the times indicated. Eight rats were used in the study. $P < 0.05$ LY83583 versus control by two-factor analysis of variance for aortic segments and kidney cortical slices.

Table IV. Time Course of cGMP Production *In Vitro* with and without LY83583 in the Presence of IBMX[a]

	fmol/mg protein		
	5 min	10 min	20 min
Aortic segments			
Control	3102 ± 1085	2541 ± 817	3390 ± 2198
LY83583	870 ± 238	514 ± 157	642 ± 142
Kidney cortical slices			
Control	579 ± 197	356 ± 18	693 ± 118
LY83583	211 ± 37	186 ± 26	202 ± 50

[a] Values are mean ± SE. Aortic segments and kidney cortical slices were incubated either with or without LY83583 (10^{-5} *M*) in the presence of 10^{-4} *M* IBMX. Four rats were used in the study. $P < 0.05$ LY83583 versus control by two-factor analysis of variance for aortic and kidney cortical slices.

in cGMP content (Ringer's solution alone: 596.5 ± 144.7 fmol/mg protein, $n = 6$ rats; LY83583: 813.7 ± 338.6 fmol/mg protein, $n = 5$ rats; $P > 0.05$).

Effect of LY83583 on the Hypotensive Response to Sodium Nitroprusside and Methacholine. Administration of vehicle alone (50% DMSO) attenuated the hypotensive action of sodium nitroprusside and isoproterenol, but not of methacholine (Table II). LY83583, in the doses given, failed to attenuate further the hypotensive response to these agents. Baseline mean arterial pressure rose from 112 ± 3 to 117 ± 3 mm Hg and from 111 ± 3 to 122 ± 3 mm Hg following vehicle and LY83583 infusion, respectively. The rise in arterial pressure produced by LY83583 (11 ± 2 mm Hg) was significantly greater than that produced by vehicle (5 ± 2 mm Hg, $P < 0.05$).

***In Vitro* Incubation of Tissues.** LY83583 (10^{-5} *M*) significantly lowered basal production of cGMP in aortic segments at 5, 10, and 20 min when factored per milligram of wet weight and when factored per milligram of protein (Table III). A concentration of 10^{-5} *M* LY83583 significantly lowered basal production of cGMP in renal cortical slices at 5, 10, and 20 min (Table III). Basal levels of cGMP in aortic and kidney tissues were severalfold greater in the presence of IBMX at 5, 10, and 20 min (Table IV). Addition of IBMX, however, did not prevent LY83583 from reducing cGMP production in either aortic segments or kidney cortical slices. In fact, LY83583 lowered cGMP to a greater extent in the presence of IBMX (compare Tables III and IV).

Discussion

Infusion of LY83583 (1-mg/kg bolus; 3 mg/kg·hr) in 75% DMSO modestly decreased plasma levels of

cGMP by 36% in conscious rats (Table I), but did not alter aortic or kidney cGMP when these organs were subsequently removed from the animals. Infusion of LY83583 (2-mg/kg bolus; 6 mg/kg·hr) produced a further decrease (46%) in plasma cGMP, but also failed to alter cGMP in kidney tissues.

Functional studies were conducted in order to determine the efficacy of LY83583 *in vivo* (Table II). In dosages up to 10 mg/kg of intravenous bolus, followed by a 6 mg/kg·hr infusion, hypotensive responses to methacholine, sodium nitroprusside, and isoproterenol were unaffected. Because these agents are believed to produce hypotension by stimulating vascular production of cGMP (2), our results with LY83583 suggest that the drug, at least in the doses administered, did not inhibit vascular cGMP production *in vivo*. The doses used were well above those employed by others *in vivo* (6), and larger doses were not considered to be practical because higher concentrations of DMSO would have been needed to dissolve it. We found that high concentrations of DMSO began to disintegrate our catheters. Moreover, the vehicle apparently had its own cardiovascular effects (Table II).

LY83583, at 10^{-5} *M*, decreased levels of cGMP in aortic segments and renal cortical slices by 65% and 50%, respectively, after 10 min of incubation (Table III). Schmidt *et al.* (1) found that the same concentration of drug attenuated cGMP accumulation in cerebellum by 73%, in lung by 47%, and in ventricular myocardium by 43% after 15 min of incubation. Diamond (7) reported that 10^{-5} *M* LY83583 lowered cGMP levels by 66% in rabbit aortic rings. It was also noted that the same concentration of LY83583 could block the elevation of cGMP normally caused by acetylcholine. Similar results were observed by Macleod *et al.* (8) and by Malta *et al.* (9). The latter found that 10^{-5} *M* LY83583 lowered basal cGMP by 88% and prevented the rise in cGMP and relaxant response upon exposure to acetylcholine and sodium nitroprusside. Thus, our data are consistent with the literature in that LY83583 worked *in vitro* to reduce tissue levels of cGMP.

Treatment of aortic and kidney tissue with 10^{-4} *M* IBMX did not prevent the ability of LY83583 from lowering cGMP production (Table IV). For example after 20 min of incubation, cGMP production was reduced by 81% and 71% in aortic and kidney segments, respectively. Evidently, LY83583 did not reduce cGMP by stimulating phosphodiesterase activity. This result confirms that of previous investigators (1).

We were surprised by the dissociation between plasma and tissue levels following administration of LY83583 *in vivo*, since extracellular levels of cGMP most likely reflect intracellular production (10–13). It is possible that the drug may have lowered cGMP content in other tissues that we did not examine. Schmidt *et al.* (1) found that after chronic administration of LY83583 to guinea pigs, cGMP was reduced in splenic, but not in lung tissue. On the other hand, platelets may be most affected by intravenous administration of LY83583 because of their intravascular location. Platelets are a rich source of soluble guanylate cyclase (14), and collagen, ADP, and thrombin (15) as well as endothelium-derived relaxant factor (16) can stimulate the enzyme. Consistent with this, Mülsch *et al.* (17) found that *in vitro* incubation of platelets with LY83583 decreased cGMP formation and facilitated platelet activation.

It is conceivable that adequate plasma concentrations of LY83583 may not have been obtained to modify cGMP production by aortic and kidney tissues. We estimate, however, that after a 2-mg/kg bolus, assuming immediate distribution and no breakdown of the compound, plasma concentrations were approximately 2×10^{-5} *M*—a concentration we found to be more than sufficient to reduce aortic and kidney cGMP levels, at least *in vitro* (Tables III and IV). Of course, if LY83583 is rapidly metabolized to inactive products, then this calculated value may be an overestimate. Alternatively, if the drug is significantly bound to plasma proteins, then free concentrations may be inadequate to affect production of tissue cGMP *in vivo*.

Finally, it is possible that DMSO, the vehicle in which LY83583 was administered, may have exerted a stimulatory effect on tissue cGMP, thereby offsetting the action of LY83583. In order to test this hypothesis, we incubated rat kidney cortical slices with 0.2% DMSO; the plasma concentration we estimated would exist if the total amount of DMSO administered to the animal was distributed in the total body water space. Kidney cortical cGMP levels were not altered by incubation with 0.2% DMSO in the presence of 10^{-4} *M* IBMX for 20 min (control = 470.2 ± 91.3 fmol/mg protein vs 0.2% DMSO = 465.4 ± 98.6 fmol/mg protein). In addition, DMSO alone did not increase plasma levels of cGMP (Table I).

The mechanism by which LY83583 reduces cGMP, at least *in vitro*, remains uncertain. It does not appear to inhibit guanylate cyclase directly (1). In fact, LY83583 actually stimulated guanylate cyclase activity in a cell-free (40,000*g* supernatant) fraction of guinea pig lung (1). The drug apparently does not reduce cGMP levels via stimulation of phosphdiesterase (Table IV) (1). Stimulation of cellular cGMP efflux is a possibility (18) which in turn would reduce the intracellular content. If, however, LY83583 augmented cellular efflux, then one might expect to see an increase in plasma levels of cGMP, not a decrease as we observed in this study (Table I). Finally, Mülsch *et al.* (19) suggested that in platelets, LY83583 may act to decrease cGMP by intracellular generation of superoxide anion which

either inactivates nitric oxide and/or induces an alteration in the enzyme guanylate cyclase.

LY83583 modestly reduced plasma levels of cGMP when infused into conscious rats; however, in our experiments, LY83583 did not lower vascular and renal cGMP when infused *in vivo,* nor did it attenuate the hypotensive response to sodium nitroprusside and methacholine. Our data do, however, confirm that LY83583 is a useful agent to lower tissue levels of cGMP *in vitro.* The mechanism(s) by which LY83583 produces this effect remains uncertain, although our results, which corroborate those of previous investigators (1), indicate that it is not through stimulation of phosphodiesterase activity.

Portions of this work appeared in abstract form (FASEB J **2**:A1066, 1988).

This project was supported by funds from Grants NIH HL38076, HD00662, and DK07301. Dr. Conrad is the recipient of the 8th Mallinckrodt Scholar Award.

1. Schmidt MJ, Sawyer BD, Truex LL, Marshall WS, Fleisch JH. LY83583: An agent that lowers intracellular levels of cyclic guanosine 3′,5′-monophosphate. J Pharmacol Exp Ther **232**:764–769, 1985.
2. Murad F. Cyclic guanosine monophosphate as a mediator of vasodilation. J Clin Invest **78**:1–5, 1986.
3. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **72**:248–254, 1976.
4. Harper JF, Brooker G. Fentomole sensitive radioimmunoassay for cyclic AMP and cyclic GMP after 2′-*O*-acetylation by acetic anhydride in aqueous solution. J Cyclic Nucleotide Res **1**:207–218, 1975.
5. Conrad KP, Vernier KA. Plasma level, urinary excretion, and metabolic production of cGMP during gestation in rats. Am J Physiol **257**:R847–R853, 1989.
6. Fleish JH, Haish KD, Spaethe SM, Rinkema LE, Cullinan GJ, Schmidt MJ, Marshall WS. Pharmacologic Analysis of two novel inhibitors of leukotriene (slow reacting substance) release. J Pharmacol Exp Ther **229**:681–689, 1984.
7. Diamond J. Effects of quinacrine, NDGA and LY83583 on acetylcholine induced elevation of cyclic GMP and relaxation of rabbit aortic rings. Proc West Pharmacol Soc **29**:105–109, 1986.
8. Macleod KM, Ng DDW, Harris KH, Diamond J. Evidence that cGMP is the mediator of endothelium-dependent inhibition of contractile responses of rat arteries to α-adrenoceptor stimulation. Mol Pharmacol **32**:59–64, 1987.
9. Malta E, Macdonald PS, Dusting GJ. Inhibition of vascular smooth muscle relaxation by LY83583. Naunyn Schmiedebergs Arch Pharmacol **337**:459–464, 1988.
10. Broadus AE, Hardman JG, Kaminsky NI, Ball JH, Sutherland EW, Liddle GW. Extracellular cyclic nucleotides. Ann NY Acad Sci **185**:50–66, 1971.
11. Hardman JG, Davis JW, Sutherland EW. Effects of some hormonal and other factors on the excretion of guanosine 3′,5′-monophosphate and adenosine 3′,5′-monophosphate in rat urine. J Clin Invest **244**:6354–6362, 1969.
12. Rosman PM, Agrawal R, Goodman AD, Steiner AL. Effect of angiotension II on cyclic guanosine monophosphate and cyclic adenosine monophosphate in human plasma. J Clin Endocrinol Metab **42**:531–536, 1976.
13. Stasch J-P, Hirth C, Kazda S, Wohlfeil S. The elevation of cyclic GMP as a response to acute hypervolemia is blocked by a monoclonal antibody directed against atrial natriuretic peptides. Eur J Pharmacol **129**:165–168, 1986.
14. Böhme E, Jung R, Mechler I. Guanylate cyclase in human platelets. Methods Enzymol **38**:199–202, 1974.
15. Nimpf J, Gries A, Wurm H, Kostner GM. Influence of β_2-glycoprotein-I upon the content of cAMP and cGMP in human blood platelets. Thrombosis Haemostasis **54**:824–827, 1985.
16. Busse R, Lückhoff A, Bassenge E. Endothelium-derived relaxant factor inhibits platelet activation. Naunyn Schmiedebergs Arch Pharmacol **336**:566–571, 1987.
17. Mülsch A, Lückhoff A, Pohl U, Busse R, Bassenge E. LY83583 (6-anilino-5,8-quinolinedione) blocks nitrovasodilator-induced cyclic GMP increases and inhibition of platelet activation. Naunyn Schmiedebergs Arch Pharmacol **340**:119–125, 1989.
18. Tjörnhammar ML, Lazaridis G, Bartfai T. Cyclic GMP efflux from liver slices. J Biol Chem **258**:6882–6886, 1983.
19. Mülsch A, Busse R, Liebay S, Förstermann U. LY83583 interferes with the release of endothelium-derived relaxing factor and inhibits soluble guanylate cyclase. J Pharmacol Exp Ther **247**:283–288, 1988.