Biochemical Pharmacology, Vol. 40, No. 10, pp. 2315–2321, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

A STRUCTURALLY NOVEL INHIBITOR OF cGMP PHOSPHODIESTERASE WITH VASODILATOR ACTIVITY

ROBERT F. G. BOOTH*, DAVID O. LUNT, NAGIN LAD, SUSAN P. BUCKHAM, SARAH OSWALD, DAVID P. CLOUGH, CHRISTOPHER D. FLOYD and JON DICKENS

Searle Research and Development, Lane End Road, High Wycombe, Buckinghamshire HP12 4HL, U.K.

(*Received* 17 *May* 1990; *accepted* 23 *July* 1990)

Abstract—A novel, potent, competitive inhibitor of smooth muscle cGMP phosphodiesterase is described (Compound I, [4-[2-*n*-butyl-5-chloro-1-(2-chlorobenzyl)imidazolyl]methyl] acetate). The compound is highly selective for inhibiting cGMP phosphodiesterase compared with cAMP phosphodiesterase. Compound I inhibits the contraction of smooth muscle in response to a variety of agonists in the same concentration range to that which inhibits the enzyme. Compound I produced a dose-related reduction in the pressor responses to angiotensin II infusion while not inhibiting the responses to bolus doses of angiotensin II. Two structural analogues of Compound I which did not inhibit cGMP phosphodiesterase failed to inhibit smooth muscle contraction *in vitro* and did not affect angiotensin II pressor responses *in vivo*. We propose a mechanism to account for the effects of a cGMP phosphodiesterase inhibitor on smooth muscle contraction *in vitro* and *in vivo*.

Guanosine cyclic 3′,5′-monophosphate (cGMP) and adenosine cyclic 3′,5′-monophosphate (cAMP) are synthesized within most mammalian cells from the corresponding nucleoside triphosphates by their respective adenylate or guanylate cyclase. Degradation of the cyclic nucleotides operates by hydrolytic cleavage of the 3′-ribose-phosphate bond, catalysed by cyclic nucleotide phosphodiesterases. The influence of cAMP on smooth muscle contraction is well documented; thus, an elevation of intracellular cAMP results in the activation of a cAMP-dependent protein kinase and protein phosphorylation which is thought to be associated with muscle relaxation [1–3]. Elevation of cAMP and smooth muscle relaxation may be elicited through direct or indirect stimulation of adenylate cyclase by agents such as β-agonists and prostacyclin, or by inhibition of cAMP phosphodiesterase. The role of cGMP in smooth muscle is less clear, although evidence suggests that the increase in cellular cGMP in smooth muscle resulting from activation of guanylate cyclase by either nitroglycerine or nitroprusside, initiates processes which lead to vascular relaxation [4, 5]. Similarly, an elevation in cellular cGMP has been suggested to account for the vasodilation caused by both atrial natriuretic peptides [6, 7] and endothelium derived relaxation factor (EDRF) [8–10]. cAMP phosphodiesterase inhibitors have been shown to cause both vascular smooth muscle relaxation and a consequent reduction in blood pressure in animal models [11]. Accumulation of intracellular cGMP resulting from inhibition of cGMP phosphodiesterase has been correlated with smooth muscle relaxation in tissue strips [12, 13].

In this report we describe the activity of a new class of compound which is a potent and selective inhibitor of cGMP phosphodiesterase. We show that the compound inhibits the contractions of smooth muscle induced by several agonists and also demonstrates antihypertensive actions *in vivo*.

* Correspondence to: Dr R. F. G. Booth, Department of Biology, Roche Products Limited, P.O. Box 8, Welwyn Garden City, Hertfordshire AL7 3AY, U.K.

MATERIALS AND METHODS

Preparation of phosphodiesterase enzymes from bovine renal artery. Bovine renal arteries and their medullary branches were obtained from slaughterhouse material and were homogenized at 4° using an Ultraturrax disintegrator (10 × 10 sec) and a glass homogenizer in a medium containing: 50 mM Tris–HCl, pH 7.4, 5 mM $MgCl_2$, 3.75 mM mercaptoethanol, 0.2 mM phenylmethylsulphonyl fluoride and 35% ethylene glycol. The homogenate was sonicated in an MSE Soniprep for 1 min (4 × 15 sec, maximal setting) and then centrifuged at 100,000 *g* for 45 min. The supernatant (approx. 35 mL) was applied to a DEAE cellulose (Sephacel) column (bed volume approx. 80 mL) and was eluted with a linear gradient of 0–1000 mM sodium acetate in a total gradient volume of 500 mL. Fractions (5 mL) were collected for subsequent phosphodiesterase assay.

Cyclic nucleotide phosphodiesterase assay. Column fractions from the chromatographed bovine renal artery were assayed to identify the peaks of enzyme activity. After assay, the individual separated peaks were pooled and compounds were assayed for inhibitory activity against the isolated phosphodiesterase isoenzymes. The enzyme assay measured the formation of 5′-[^{3}H]AMP or 5′-[^{3}H]GMP from the corresponding 3′,5′-cyclic nucleotides. The 5′-nucleotide was then converted by a 5′-nucleotidase from snake venom *(Ophiophagus hannah)* to the equivalent nucleoside which was then isolated by anion exchange chromatography [14]. Assays were performed at 1 μM substrate concentration, at 30°, for 10 min using enzyme dilutions which gave 20–

30% hydrolysis of substrate. IBMX was included in each series of assays to ensure that the enzyme preparation was functioning in a standardized manner. The enzyme incubation mixture contained 50 mM Tris–HCl, pH 8.0, 30 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.1 mg/mL bovine serum albumin, 1 μM cyclic 3′,5′-[^{3}H]AMP or cyclic 3′,5′-[^{3}H]GMP (5×10^5 cpm) and an appropriate dilution of enzyme in a volume of 0.5 mL. In enzyme inhibition assays, compounds were dissolved in DMSO and always added to the assay to give a final concentration of 2% DMSO. Each assay was initiated by addition of substrate and was terminated by boiling for 1 min followed by a further 10 min incubation with 50 μL snake venom (2 mg/mL) at 30°. Methanol (1 mL) was then added and the mixture added to a small anion exchange column (AG1 X-8, 0.6×2 cm column). [^{3}H]Adenosine or [^{3}H]guanosine were eluted by the addition of a further 1 mL of methanol directly into scintillation vials. Both control and non-enzyme blank assays were measured in the presence of 2% DMSO. The enzyme assay was linear for at least 20 min and none of the compounds investigated affected the activity of the 5′-nucleotidase. The concentrations of compounds which inhibited by 50% the hydrolysis of 1 μM substrate (IC_{50}) were determined from concentration per cent curves. Determination of inhibition constants was carried out by selecting a grid of substrate and inhibitor concentrations which spanned the K_m and approximated K_i values for the enzyme.

Inhibition of smooth muscle contraction. Male New Zealand White rabbits (1.5–2.5 kg) were killed by cervical dislocation. The mesenteric arteries were removed carefully to protect the endothelial lining and transferred to plastic dishes containing Krebs–Ringer bicarbonate buffer: 117 mM NaCl, 4.7 mM KCl, 1.1 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 1.5 mM $CaCl_2$ and 20 mM glucose. The vessels were cleaned of adherent tissues and carefully cut into helical strips which were 3 mm wide and 10–12 mm long. The strips were transferred to a 10 mL organ bath containing warm (37°), oxygenated (95% O_2, 5% CO_2) Krebs solution. After 30 min the tissues were stretched with 2.0 g. Tension changes were recorded isometrically with Lectromed force displacement transducers on Lectromed MX216 Polygraphs. The tissues were allowed to equilibrate for a minimum of 90 min prior to drug additions. Agonists were added to the organ bath in sufficient concentration to cause 60% of maximal force of contraction and this was established by successive addition and wash-out of increasing concentrations of agonist. Tissues were assessed for endothelial integrity by the capacity of acetylcholine to induce relaxation. After a baseline contraction had been established, inhibitor was added to the relaxed strip and allowed to equilibrate for 15 min prior to addition of agonist. After addition of agonist, the percentage inhibition of force of contraction was calculated and the inhibitor washed out. This procedure was repeated at successively increasing concentrations of inhibitor to allow an IC_{50} to be calculated from the curves of percentage inhibition versus log inhibitor concentration. Usually four strips were prepared at one time.

Measurement of blood pressure responses in anaesthetized rats. Female Wistar rats (240–260 g body wt) were anaesthetized with Inactin (160 mg/kg, i.p.). Body temperature was maintained at 37°. Arterial blood pressure was measured via the carotid artery using a pp50 polythene cannula connected to a Lectromed Type 4 422-0001-1-B4M5 pressure transducer and Lectromed MT6 multi-channel recorder. The jugular vein was cannulated for simultaneous drug injection and where appropriate, continuous angiotensin II infusion. Experiments were carried out in three ways.

(1) In the first series of experiments the effect of test compounds on the resting blood pressure of normotensive anaesthetized rats was examined. After cannulation, the blood pressure was monitored until it had remained stable for at least 15 min. Test compounds were then infused over a period of 5 min in a total volume of 1 mL after being dissolved in a vehicle of PEG 200–ethanol–saline (4:1:5, by vol.). Blood pressure was monitored for a period of 60 min after infusion.

(2) In the second series of experiments the effect of test compounds on the pressor responses to bolus doses of angiotensin II and noradrenaline were monitored. In these experiments, angiotensin II (100 ng/kg) and noradrenaline (100 ng/kg) were successively injected at 3 min intervals until at least three similar consecutive pressor responses had been achieved for each pressor agent. Test compounds were then infused over a period of 5 min in a total volume of 1 mL after being dissolved in a vehicle of PEG 200–ethanol–saline (4:1:5, by vol.). Pressor responses to bolus doses of angiotensin II (100 ng/kg) were then monitored at 2, 10, 20 and 30 min after test compound infusion and to bolus doses of noradrenaline (100 ng/kg) at 5 and 30 min after test compound infusion. Results were expressed in terms of the pressor responses to angiotensin II and noradrenaline before and after administration of the test compound. Infusion of vehicle alone had no effect on the pressor response to either angiotensin II or noradrenaline.

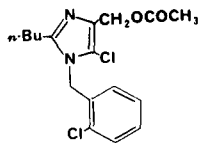
(3) In the third series of experiments the effects of test compounds on the pressor responses to a continuous infusion of angiotensin II were monitored. Angiotensin II was suspended in normal saline (1 μg/mL) and infused at a rate of 0.1 mL/min (100 ng/kg/min) using a Braun infusion pump. After blood pressure had stabilized (usually an approximate 30 mm Hg elevation in mean arterial blood pressure) test compound was infused as described above. The influence of infusion of the test compound on the pressor responses to angiotensin II infusion was monitored for 60 min. Injection of the vehicle alone had no significant effect on the blood pressure. Mean arterial blood pressure (MABP) was calculated from the equation:

$$\text{MABP} = \text{Diastolic blood pressure} + \frac{(\text{systolic blood pressure} - \text{diastolic blood pressure})}{3}.$$

Heart rate was constantly monitored in all animals using HR2 heart rate meters (Autoradiographic

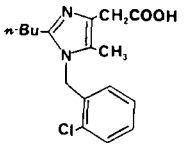
COMPOUND I

4 - (2 - n - Butyl - 5 - chloro - 1 - (2 - chlorobenzyl) imidazolyl) methyl acetate



COMPOUND II

4 - (2 - n - Butyl - 1 - (2 - chlorobenzyl) - 5 - methyl) imidazoleacetic acid



COMPOUND III

4 - (2 - n - Butyl - 5 - chloro -1 - (2 - chlorobenzyl)) imidazoleacetic acid

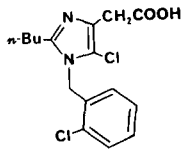


Fig. 1. Compounds I–III.

Products, Cheshire, U.K.) which were connected to a Lectromed MT6 multi-channel recorder.

Materials. The following compounds were obtained commercially: phenylmethylsulphonyl fluoride, calmodulin, snake venom 5′-nucleotidase *(Ophiophagus hannah)*, angiotensin II and noradrenaline (Sigma Chemical Co., Poole, U.K.). Methoxamine (Burroughs Wellcome, NC, U.S.A.), DEAE Sephacel (Pharmacia, Uppsala, Sweden). DEAE anion exchange resin AG 1-X8, 200–400 mesh, chloride form (Bio-Rad Laboratories, Watford, U.K.). Inactin Byk (Byk Gulden, Konstanz, F.R.G.). [^{3}H]Adenosine cyclic 3,5′-monophosphate, ammonium salt (29.4 Ci/mmol) and [^{3}H]guanosine cyclic 3,5′-monophosphate, ammonium salt (15 Ci/mmol) was obtained from Amersham International (Amersham, U.K.).

Compounds I and II were prepared using conventional methods from the appropriate 2-*n*-butyl-4-substituted imidazolemethanols [15]. Compound III was prepared according to the published method [16]. Compounds I, II and III are shown in Fig. 1.

RESULTS

Inhibition of cGMP phosphodiesterase

Using anion exchange chromatography, the cyclic nucleotide phosphodiesterase activity of bovine renal artery was resolved into three separate peaks. The first peak of enzyme activity only hydrolysed cGMP at the substrate concentration (1 μM) used in the assay. This enzyme, designated the cGMP phosphodiesterase, was stimulated 8–10-fold in the presence of calcium (10 μM) and calmodulin (6 μg/mL). Since the addition of calcium and/or calmodulin did not affect the inhibitory activity of any of the compounds examined, these components were omitted from further assays. The second peak of enzyme activity showed a lack of specificity for either cAMP or cGMP as substrate and this enzyme was not used in further studies. The third peak of enzyme activity showed a selectivity for the hydrolysis of cAMP at the substrate concentration used (1 μM) in the assay. This enzyme was designated the cAMP phosphodiesterase and its activity was unaffected by the presence or absence of calcium and/or calmodulin.

Table 1 shows the results obtained for three standard phosphodiesterase inhibitors including M&B22948, LH725 and IBMX and three imidazole derived phosphodiesterase inhibitors.

Of the compounds shown, only M&B22948 and Compound I show potent and selective inhibition of cGMP phosphodiesterase. Interestingly, M&B22948 was an order of magnitude more potent against cGMP phosphodiesterase from the bovine renal artery enzyme than has been previously reported for the same enzyme from bovine coronary artery (IC_{50} 0.39 μM cf. 2.4 μM) [17]. Under the conditions of the present assay, Compound I represents one of the most potent calcium/calmodulin sensitive cGMP phosphodiesterase inhibitors yet reported. Under the same assay conditions, Compound II was a weak inhibitor of the enzyme and compound III was not inhibitory at concentrations up to 100 μM.

A more detailed kinetic analysis of the inhibition of cGMP phosphodiesterase by Compound I is shown in Fig. 2. The data demonstrate that Compound I is a competitive inhibitor with a K_i of 5 μM with respect to cGMP.

Effect of Compound I on smooth muscle contraction

The concentration of agonist causing 60% of maximal contraction was established for each tissue strip and for noradrenaline was in the range 0.5×10^{-6} to 5×10^{-6} M; for angiotensin II, 1–10 ng/mL; for 5-hydroxytryptamine, 50–300 ng/mL; and for methoxamine, 50–500 ng/mL.

When Compound I was added to strips of mesenteric artery which had been pre-contracted with noradrenaline, there was no evidence of relaxation at concentrations up to 50 μM. However, when Compound I was preincubated with the tissue for 15 min prior to the addition of agonist, an inhibition of the agonist-induced contraction occurred and a summary of the results is shown in Table 2. It is clear that Compound I inhibits the contraction of rabbit mesenteric artery in response to a spectrum of agonists; however, since the degree of inhibition varied considerably with individual tissue strip preparations, a range of inhibitory EC_{50} values is also presented. The inhibitory activity of Compound I on the mesenteric artery could be completely reversed by washing the tissue three times over a period of 30 min with Krebs–Ringer bicarbonate. Compounds II and III, which are close structural analogues of Compound I, but

Table 1. Comparative activities of compounds versus bovine renal artery cGMP-dependent and cAMP-dependent phosphodiesterase

Compound	Inhibition of phosphodiesterase IC_{50} (μM) ± SE	
	cGMP-dependent PDE	cAMP-dependent PDE
Compound I	2.23 ± 0.34	>200
Compound II	61 ± 9	>200
Compound III	>100	>200
Zaprinast (M&B 22948)	0.39 ± 0.34	230*
Trequinsin (HL 725)	0.67 ± 0.30	0.21 ± 0.03
Isobutylmethylxanthine (IBMX)	4.58 ± 0.17	6.31 ± 0.55

* Data from human lung phosphodiesterase [38].

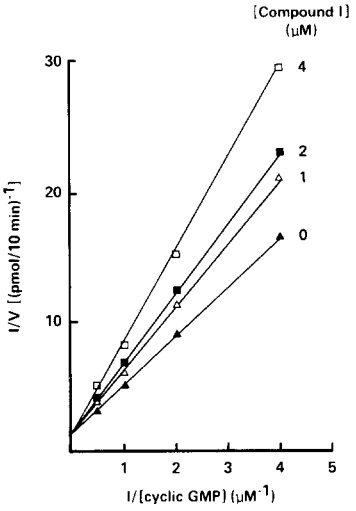


Fig. 2. Competitive nature of the inhibition of bovine renal artery cGMP-dependent PDE I by Compound I. Initial rates of the PDE activities from DEAE Sepharose chromatography were measured at various cyclic GMP concentrations in the presence of 4 μM (□), 2 μM (■), 1 μM (△) or no (▲) inhibitor.

Table 2. Inhibition of smooth muscle contraction by Compound I

Agonist	Inhibition of contraction EC_{50} (μM)
Noradrenaline	6 (Range 5–10 μM)
Angiotensin II	10 (Range 5–15 μM)
5-Hydroxytryptamine	20 (Range 10–30 μM)
Methoxamine	10 (Range 3–15 μM)

Data shown represents the mean of at least four determinations on individual preparations of rabbit mesenteric artery from different animals.

which possess only weak to negligible activity against cGMP phosphodiesterase (Table 1), did not inhibit the contraction of mesenteric artery in response to any agonist; this implies a correlation between inhibition of smooth muscle cGMP phosphodiesterase and inhibition of smooth muscle contraction.

Effect of Compounds I, II and III on blood pressure

In the first group of experiments where the effects of the test compounds on resting blood pressure in anaesthetized normotensive rats were examined, administration of Compound I (10 mg/kg, i.v.) caused a sustained 20–30 mmHg fall in blood pressure over the monitoring period of 60 min (data not shown). Infusion of vehicle alone caused a small (8 mmHg) and transitory fall in blood pressure which was not significant after 60 min. Infusion of either Compound II or Compound III had no effect on the blood pressure above that seen with vehicle alone.

In the second group of experiments, the effects of Compounds I, II and III on the elevation of mean arterial blood pressure evoked by bolus doses of angiotensin II and noradrenaline were investigated. After consistent pressor responses had been obtained for angiotensin II and noradrenaline, Compounds I, II and III (all at 10 mg/kg, i.v.) were infused over a period of 5 min. After blood pressure responses had remained stable for a period of 5 min, bolus doses of angiotensin II and noradrenaline were then injected at regular intervals up to 1 hr after drug infusion. None of the compounds influenced the pressor responses to either angiotensin II or noradrenaline.

In the third group of experiments, the influence of Compounds I, II and III (all 10 mg/kg i.v.) on the elevated mean arterial blood pressure evoked by a continuous infusion of angiotensin II were monitored. Compounds II and III had no effect on the angiotensin II pressor response. However, Compound I produced a dose-related reduction in mean arterial blood pressure (Fig. 3). One minute after infusion of Compound I (10 mg/kg) the mean arterial blood pressure was reduced by 43 mmHg although this reduction in the angiotensin II pressor response had disappeared by 60 min. Infusion of Compound I did not cause a statistically significant change in heart rate.

DISCUSSION

The results described in this report demonstrate that Compound I is a competitive inhibitor of the calmodulin-dependent cGMP phosphodiesterase derived from bovine renal artery with a K_i of 5 μM. The inhibitors are amongst the most potent yet

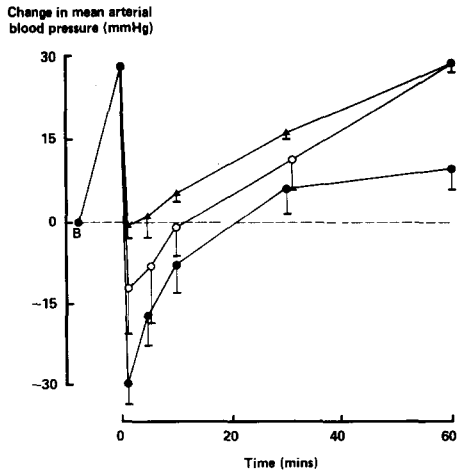


Fig. 3. The effect of Compound I on the pressor responses to angiotensin II in the anaesthetized rat. Blood pressure responses are shown as changes from basal (B) mean arterial blood pressure which in this series of experiments was 106.6 ± 6.1 mmHg (N = 12). At point B angiotensin II was infused at a rate of 100 ng/min and infusion was continued throughout the study. By 30 min blood pressure stabilized (elevation of 29.1 ± 1.8 mmHg (N = 12). At this point ($t = 0$), Compound I was administered and blood pressure responses and heart rate were monitored for a further 60 min. Compound I was infused to three different final concentrations, 30 mg/kg (●), 10 mg/kg (○) and 3 mg/kg (▲). Results are shown with error bars of ±SE and pressor responses were monitored in at least three different animals for each drug concentration.

described for cGMP phosphodiesterase and are also highly selective for cGMP phosphodiesterase over cAMP phosphodiesterase. Only M&B22948 [17] and a series of dibenzoquinazoline diones have been reported to possess similar potency and selectivity [18].

The chromatographic procedures utilized in the present experiments allow resolution of isoenzymes which are relatively selective for cGMP hydrolysis from those selective for cAMP hydrolysis. A comparison with alternative chromatographic separations indicates that the cGMP-dependent phosphodiesterase fraction could include both Ca^{2+}/calmodulin-sensitive and -insensitive cGMP-dependent isoenzymes [19, 20]. However, although the addition of calcium/calmodulin resulted in an increase in enzyme specific activity, there was no effect on the inhibitory activity of the compounds examined, suggesting either that only the calcium/calmodulin-sensitive isoenzyme was present, or that all of the compounds tested were equally inhibitory towards both calcium/calmodulin-sensitive and -insensitive isoenzymes.

Of the compounds investigated in the present study, a potent inhibition of cGMP phosphodiesterase correlated with the ability to inhibit smooth muscle contraction in response to a spectrum of agonists. Moreover, the concentration of Compound I required to inhibit cGMP phosphodiesterase was in the same range as that which functionally inhibited the contraction of arterial smooth muscle. A similar correlation between the potency of inhibition of tracheal smooth muscle cGMP phosphodiesterase and tracheal smooth muscle relaxation for four alkylxanthines has been reported [13].

Sodium nitroprusside, nitroglycerine and isosorbide 5′-mononitrate all cause smooth muscle relaxation *in vivo* and they are used clinically as vasodilator agents in the treatment of angina and uncontrolled acute hypertension [21–23]. To assess the potential vasodilator properties of Compound I *in vivo* it was examined in three different anaesthetized rat models. In the second of these models the ability of bolus doses of Compounds, I, II and III to influence the pressor effects of bolus doses of angiotensin II and noradrenaline was determined. None of the three compounds examined reduced the pressor responses. In both the third model, which utilized a continuous infusion of angiotensin II to induce a sustained hypertension, and the first model which was a normotensive anaesthetized rat preparation, neither Compound II nor III influenced blood pressure, while Compound I produced a dose related reduction in the pressor response. Therefore, while transient pressor responses are resistant to Compound I, both resting blood pressure and also the prolonged pressor responses resulting from an infusion of angiotensin II were sensitive to Compound I. Thus, comparing the second and third models, the sensitivity of the response to Compound I was dependent on the rate of administration of angiotensin II and the inhibitory effect was not observed if bolus doses of the pressor agent were used. Infusions of angiotensin II may be considered to reflect more closely the physiological events which govern smooth muscle contraction *in vivo*. Similar *in vivo* responses to those observed with Compound I have been reported for the inhibition of pressor responses to angiotensin II by nifedipine, where bolus doses of angiotensin II were unaffected by the presence of nifedipine, while the pressor response to angiotensin II infusion was much reduced [24]. This observation was taken to indicate that in angiotensin II infusion a substantial component of the contraction was dependent on calcium entry.

The results from the *in vivo* experiments using Compounds I–III correlate with the isolated tissue experiments and suggest that there is also a correlation with the potency of these compounds as cGMP phosphodiesterase inhibitors. It should be recognized that each of these series of experiments was carried out using a different species, i.e. bovine renal artery, rabbit mesenteric artery and *in vivo* in the rat [19]. However, Compounds I–III demonstrated a similar profile of cGMP phosphodiesterase inhibitory activity for isoenzymes which were isolated from both the human platelet and the rat heart using a methodology similar to that used for isolation of the bovine renal artery isoenzymes (data not shown).

A role for cGMP in mediating contractile events was proposed more than a decade ago [25]. In contrast to agents such as nitroprusside and nitroglycerine which directly stimulate guanylate cyclase, a cGMP phosphodiesterase inhibitor requires either a continuous basal rate of generation of cGMP

within the cell or an agonist stimulation of guanylate cyclase to raise tissue cGMP concentrations. Thus, the cGMP phosphodiesterase inhibitor, M&B22948, causes only a small elevation in tissue cGMP content and slight relaxation of smooth muscle, but both of these effects are greatly potentiated in the presence of nitroglycerine [26].

A number of biochemical mechanisms could explain the capacity of cGMP to cause relaxation of smooth muscle; however, these may be broadly divided into those mechanisms which explain the vasorelaxation by an effect on intracellular Ca^{2+} concentration and those which provide alternative mechanisms.

The mechanism by which cGMP influences cellular Ca^{2+} concentration is not fully elucidated, but it has been demonstrated that the calcium entry which is responsible for the tonic contraction of smooth muscle occurs through receptor mediated calcium channels [27] and also that the gating of these channels, by noradrenaline is sensitive to cGMP [28]. Thus, in both the normotensive rat and the rat model of angiotensin II infusion a constant stimulation of the angiotensin II receptor may be maintaining the calcium channels in an open state to allow a tonic contraction. Addition of a cGMP phosphodiesterase inhibitor may inhibit the calcium gating thereby reducing the sustained tonic contraction in resistance vessels with a consequent reduction in blood pressure.

Elevation of cGMP has also been shown to increase the intracellular sequestration of Ca^{2+} and to stimulate the efflux of intracellular Ca^{2+}. When cGMP is elevated in cells, cGMP-dependent protein kinase is activated which can phosphorylate phospholamban and thereby stimulate Ca^{2+} uptake by the sarcoplasmic reticulum of cardiac and smooth muscle [29, 30]. Efflux of Ca^{2+} from cells can be increased via cGMP through stimulation of cGMP-dependent protein kinase and a consequent activation of the ATP-dependent Ca^{2+} pump of the plasmalemma [31, 32]. Furthermore, a cGMP-dependent protein kinase stimulation of the $(Ca^{2+} + Mg^{2+})$-dependent ATPase of the plasmalemma has also been reported [33, 34]. It has recently been suggested that the effect may be mediated through an indirect effect by increasing phosphatidylinositol phosphate which can directly stimulate the $(Ca^{2+} + Mg^{2+})$-ATPase [35].

The decrease in intracellular Ca^{2+} caused by an elevation of cGMP may also decrease the activity of myosin light chain kinase (MLCK) which thereby reduces the extent of phosphorylation of the myosin light chain and decreases tension development [36]. Additionally, cGMP appears to cause a decrease in the sensitivity of the contractile elements of smooth muscle cells to Ca^{2+} through a pathway which is probably mediated by protein kinases [37].

REFERENCES

1. Kramer GL and Hardman JG, Vascular smooth muscle. In: *Handbook of Physiology: The Cardiovascular System* (The American Physiological Society, Bethesda), pp. 179–199, 1980.
2. Miller JR, Silver PJ and Stull JT, The role of myosin light chain kinase phosphorylation in beta-adrenergic relaxation of tracheal smooth muscle. *Mol Pharmacol* **24**: 235–242, 1983.
3. Demaille JG and Pechere JP, The control of contractility by protein phosphorylation. *Adv Cyclic Nucleotide Res* **15**: 337–371, 1983.
4. Diamond J and Blisard KS, Effects of stimulant and relaxant drugs on tension and cyclic nucleotide levels in canine femoral artery. *Mol Pharmacol* **12**: 688–692, 1976.
5. Murad F, Rapoport RM and Fiscus R, Role of cyclic-GMP in relaxations of vascular smooth muscle. *J Cardiovasc Pharmacol* **7** (Suppl 3): S111–S118, 1985.
6. Hamet P, Trembley J, Pang SC, Garcia R, Thibault G, Gutowska J, Cantin M and Genest J, Effect of native and synthetic atrial natriuretic factor on cyclic GMP. *Biochem Biophys Res Commun* **123**: 515–527, 1984.
7. Fiscus RR, Rapoport RM, Waldmann SA and Murad F, Atriopeptin II elevates cyclic GMP, activates cyclic GMP-dependent protein kinase and causes relaxation in rat thoracic aorta. *Biochim Biophys Acta* **846**: 179–184, 1985.
8. Mellion BT, Ignarro LJ, Ohlstein EH, Pontecorvo EG, Hyman AL and Kadowitz PJ, Evidence for the inhibitory role of cyclic GMP in ADP-induced human platelet aggregation in the presence of nitric oxide and related vasodilators. *Blood* **57**: 946–955, 1981.
9. Furchgott RF and Jothiandan D, Regulation of cGMP levels to endothelium dependent relaxation by acetylcholine in rabbit aorta. *Fed Proc* **42**: 619, 1983.
10. Rapoport RM and Murad F, Agonist-induced endothelium-dependent relaxation in rat thoracic aorta may be mediated through cyclic GMP. *Circ Res* **52**: 352–357, 1983.
11. Lal B, Dhadwalla AN, Dadkar NK, D'Sa A and DeSouza NJ, Trequinsin, a potent new antihypertensive vasodilator in the series of 2-(Arylimino)-3-alkyl - 9,10 - dimethyoxy - 3,4,6,7 - tetrahydro - 2H - pyrimido[6,1-a]-isoquinolin-4-ones. *J Med Chem* **27**: 1470–1480, 1984.
12. Kramer GL and Wells JN, Effects of phosphodiesterase inhibitors on cyclic nucleotide levels and relaxation of pig coronary arteries. *Mol Pharmacol* **16**: 813–822, 1979.
13. Polson JB, Krzanowski JJ and Szentivanyi A, Correlation between inhibition of a cyclic GMP phosphodiesterase and relaxation of canine tracheal smooth muscle. *Biochem Pharmacol* **34**: 1875–1879, 1985.
14. Thompson WJ, Terasaki WL, Epstein PM and Strada SJ, Assay of cyclic nucleotide phosphodiesterase and resolution of multiple molecular forms of the enzyme. *Adv Cycl Nucl Res* **10**: 69–92, 1979.
15. Furukawa Y, Kishimoto S and Nishikawa K, Hypertensive imidazole derivatives. US Patents 4,300,598, July 20 and 4,355,040, October 19, 1982.
16. Furukawa Y, Kishimoto S, Nishikawa K, 1984. European Patent 0,028,834, January 18, 1984.
17. Weishaar RE, Burrows SD, Kobylarz DC, Quade MM and Evans DB, Multiple molecular forms of cyclic nucleotide phosphodiesterase in cardiac and smooth muscle and in platelets. *Biochem Pharmacol* **35**: 787–800, 1986.
18. Booth RFG, Buckham SP, Lunt DO, Manley PW and Porter RA, Dibenzoquinazoline diones as antihypertensive cyclic guanosine monophosphate phosphodiesterase inhibitors. *Biochem Pharmacol* **36**: 3517–3521, 1987.
19. Lugnier C, Schoeffter P, LeBec A, Strouthou E and Stoclet JC, Selective inhibition of cyclic nucleotide phosphodiesterases of human, bovine and rat aorta. *Biochem Pharmacol* **35**: 1743–1751, 1986.

20. Reeves ML, Leigh BK and England PJ, The identification of a new cyclic nucleoside phosphodiesterase activity in human and guinea pig cardiac ventricle. *Biochem J* **241**: 535–541, 1987.
21. Roger F, Palmer MD and Lasseter KC, Sodium nitroprusside. *N Engl J Med* **292**: 294–297, 1975.
22. Fuchs RM, Brinker JA, Guzman PA, Kross DE and Yin FCP, Regional coronary blood flow during relief of pacing-induced angina by nitroglycerin. *Am J Cardiol* **51**: 19–27, 1983.
23. Uberbacher HJ, Steinorth G, Glocke M and Abshagen U, An open long-term, multi-centre study of the anti-anginal efficacy and safety of isosorbide 5-mononitrate in patients with coronary heart disease. *Pharmatherapeutica* **3**: 331–337, 1983.
24. Grant T and McGrath JC, Effects of nifedipine on pressor responses to angiotensin II in the pithed rat. *Blood vessels*. July–August, 189, 1984.
25. Dunham EW, Haddox MK and Goldberg ND, Alteration of vein cyclic 3′,5′ nucleotide concentrations during changes in contractility. *Proc Natl Acad Sci USA* **71**: 815–819, 1974.
26. Kukovetz WR, Holzmann S, Wurm A and Poch G, Evidence for cGMP-mediated relaxant effects of nitro-compounds in coronary smooth muscle. *Naunyn Schmiedebergs Arch Pharmacol* **310**: 129–138, 1979.
27. Godfraind T, Calcium entry blockade and excitation-contraction coupling in the cardiovascular system. *Acta Pharmacol Toxicol* **58** (Suppl. II): 5, 1986.
28. Godfraind T, EDRF and cyclic GMP control gating of receptor operated calcium channels in vascular smooth muscle. *Eur J Pharmacol* **126**: 341–343, 1986.
29. Twort CHC and van Breemen C, Cyclic guanosine monophosphate-enhanced sequestration of calcium by sarcoplasmic reticulum in vascular smooth muscle. *Circ Res* **62**: 961–964, 1988.
30. Raeymakers L, Hofmann F and Casteels R, Cyclic GMP-dependent protein kinase phosphorylates phospholamban in isolated sarcoplasmic reticulum from cardiac and smooth muscle. *Biochem J* **252**: 269–273, 1988.
31. Kobayashi S, Kanaide H, Nakamura M, Cytosolic-free calcium transients in cultured vascular smooth muscle cells: microfluorimetric measurements. *Science* **229**: 553–556, 1985.
32. Rashatwar SS, Cornwell TL and Lincoln TM, Effects of 8-bromo-cGMP on Ca^{2+} levels in vascular smooth muscle cells: possible regulation of Ca^{2+}-ATPase by cGMP-dependent protein kinase. *Proc Natl Acad Sci USA* **84**: 5685–5689, 1987.
33. Suematsu E, Hirata M and Kuriyama H, Effects of cAMP and cGMP-dependent protein kinases, and calmodulin on Ca^{2+} uptake by highly purified sarcolemmal vesicles of vascular smooth muscle. *Biochim Biophys Acta* **773**: 83–90, 1984.
34. Popescu LM, Foril CP, Hinescu M, Paniou C, Cinterza M and Glerasim L, Nitroglycerin stimulates the sarcolemmal Ca^{2+}-extrusion ATPase of coronary smooth muscle cells. *Biochem Pharmacol* **34**: 1857–1860, 1985.
35. Vrolix M, Raeymakers L, Wuytack F, Hofmann F and Casteels R, Cyclic GMP-dependent protein kinase stimuates the plasmalemmal Ca^{2+} pump of smooth muscle via phosphorylation of phosphatidylinositol. *Biochem J* **255**: 855–863, 1988.
36. Kamm KE and Stull JT, The function of myosin and myosin light chain kinase phosphorylation in smooth muscle. *Annu Rev Pharmacol Toxicol* **25**: 593–620, 1985.
37. Nishimura J and van Breemen C, Direct regulation of smooth muscle contractile elements by second messengers. *Biochem Biophys Res Commun* **163**: 929–935, 1988.
38. Bergstrand H, Kristofferson J, Lundquist B and Schurmann A, Effects of antiallergic compounds, compound 48/80, and some reference inhibitors on the activity of partially purified human lung tissue adenosine cyclic 3′,5′-monophosphate and guanosine cyclic 3′,5′-monophosphate phosphodiesterases. *Mol Pharmacol* **13**: 38–43, 1977.