JACC Vol. 8, No. 2
August 1986:342–8

Effect of 8-Bromo-Cyclic Guanosine Monophosphate (cGMP) on Coronary Artery Constriction in Isolated Rabbit Hearts

RICHARD J. BING, MD, FACC, MAYTHEM SAEED, PHD

Pasadena, California

The vasodilator 8-bromo-guanosine 3′:5′-monophosphate (8-bromo-cGMP) effectively counteracts vasopressin-induced coronary artery constriction in a supported perfused working rabbit heart. In this preparation, the coronary arteries remain in contact with the beating heart. The obtuse marginal artery and portions of the left anterior descending coronary artery were deprived of endothelium. Perfusion was carried out with Krebs-Henseleit solution, oxygenated with a disposable infant oxygenator. The internal diameter of large coronary arteries was determined by color arteriography (injection of patent blue dye and gated photography). The effect of vasopressin with and without the addition of 8-bromo-cGMP on cardiac performance (cardiac output, left ventricular systolic pressure, left ventricular end-diastolic pressure, maximal rate of rise in left ventricular pressure [dP/dt_{max}], mean aortic pressure) and large coronary vessel and total coronary vascular resistance was determined in nine experiments. In addition, changes in coronary sinus partial pressure of carbon dioxide (P_{CO_2}) and pH were observed.

Vasopressin alone caused a significant decline in coronary flow, myocardial oxygen consumption and coronary sinus pH. Cardiac performance declined, probably because of myocardial ischemia. Large coronary vessel and total coronary vascular resistance rose. The vasodilator 8-bromo-cGMP strongly inhibited the vasoconstrictor action of vasopressin, counteracted the increase in large and total coronary vascular resistance, prevented the fall in myocardial oxygen consumption and eliminated changes in pH or P_{CO_2} of coronary sinus effluent. Because of the elimination of myocardial ischemia by 8-bromo-cGMP, cardiac performance was normalized. The presence of 8-bromo-cGMP significantly shifted the dose-response relation between vasopressin and coronary flow and between vasopressin and total coronary resistance to the right, and caused an increase in the units for the concentration required to obtain 50% of the maximal effect. The results indicate that 8-bromo-cGMP has the potential to become a useful agent in counteracting coronary artery constriction in vivo, particularly in those instances in which the coronary endothelium has been damaged.

(J Am Coll Cardiol 1986;8:342–8)

It is recognized that guanosine 3′:5′-monophosphate (cyclic GMP) is released in arterial smooth muscle cells as a result of the action of an endothelial-derived relaxing factor (1–3) and of a series of vasodilator compounds (4–6), including nitrovasodilators and acetylcholine (1–6). The presence of vascular endothelium potentiates the release of cyclic GMP and thus mediates the relaxing effect of acetylcholine both in isolated coronary arteries (7–9) and in coronary arteries perfusing an intact heart (10). An 8-bromo analogue of cyclic GMP, 8-bromo-cGMP, first synthesized by Michal et al. (11), causes profound muscular relaxation in preconstricted isolated aortas (6,9,12). As compared with cyclic GMP, 8-bromo-cGMP possesses greater cellular penetration and is less vulnerable to the splitting action of a phosphodiesterase (11). The extent of 8-bromo-cGMP–induced decreased tension depends on the initial tension of the noradrenaline-stimulated tissue (6,13). Saeed and Bing (unpublished data, 1986) confirmed the relaxing effect of 8-bromo-cGMP on noradrenaline-preconstricted rabbit aortic strips and the dependency of the degree of relaxation on initial tension. They also found a higher degree of relaxation in rabbit aortic strips that were deprived of endothelium.

This report is concerned with the effect of 8-bromo-cGMP on endothelium-deprived coronary arteries perfusing a beating heart. Constriction of the coronary arteries was

From the Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, California. This work was supported by grants from The Council for Tobacco Research, U.S.A., Inc., New York, New York, The Margaret W. and Herbert Hoover, Jr. Foundation, Los Angeles, California and The Charles A. Lindbergh Fund, Inc., Minneapolis, Minnesota. An abstract of this paper was presented at the 35th Annual Scientific Session of the American College of Cardiology, Atlanta, Georgia, March 9 to 13, 1986.

Manuscript received December 23, 1985; revised manuscript received February 25, 1986, accepted March 19, 1986.

Address for reprints: Richard J. Bing, MD, Huntington Memorial Hospital, 100 Congress Street, Pasadena, California 91105.

©1986 by the American College of Cardiology

0735-1097/86/$3.50

accomplished with the pituitary hormone vasopressin (8-arginine vasopressin). The results demonstrate that in the absence of endothelium, 8-bromo-cGMP diminishes or abolishes vasopressin-induced large artery constriction, and abolishes the increase in total coronary resistance and the diminution in coronary flow. In the presence of 8-bromo-cGMP, the dose-response relation between vasopressin and total coronary flow and total coronary vascular resistance is shifted to the right.

Methods

Experimental preparation. Nine male albino rabbits weighing 2.1 to 2.8 kg were anesthetized with 30 mg/kg body weight sodium pentobarbital, intravenously (Abbott Laboratories) and heparinized with 400 IU/kg, intravenously. Depth of anesthesia was ascertained by lack of corneal reflexes. After tracheotomy, the rabbits were artificially ventilated with 95% oxygen and 5% carbon dioxide using a respirator (Mark 10, ventilator, Bird Corp.). Tracheotomy and ventilation were carried out to supply the heart with sufficient oxygen during thoracotomy. The heart was quickly removed and placed in ice cold saline solution. The previously outlined technique was followed in principle (13,14). The obtuse marginal coronary artery and the first centimeter of the anterior descending coronary artery were deprived of endothelium by an abraded polyethylene tubing introduced through the sinus of Valsalva. The aorta, pulmonary artery and left atrium were cannulated. These procedures were carried out with the heart submerged in chilled saline solution.

The heart was then perfused in a Langendorff preparation and the venae cavae and pulmonary veins were ligated. Krebs-Henseleit solution at 37°C equilibrated for 20 minutes with 95% oxygen and 5% carbon dioxide was used as perfusate (13,14). The heart was then quickly transferred to a modified supported working heart perfusion apparatus, again using Krebs-Henseleit solution (500 ml) as perfusate. The Krebs-Henseleit solution consisted of (in m*M*): Na^+ 143, K^+ 5.94, Ca^{2+} 2.54, Mg^{2+} 1.19, phosphate 1.19, Cl^- 128, HCO_3^- 25, SO_4^{2-} 1.2 and glucose 10; pH was maintained between 7.35 and 7.45 by adjusting the rate of bubbling of the gas mixture. Krebs-Henseleit solution was used rather than a perfluorocarbon, because the detergent, pluronic (F-68) and hydroxyethylstarch in the perfluorocarbon might interfere with the effect of vasoactive substances. Oxygenation of the perfusate was accomplished with a disposable infant blood oxygenator (Spiroflo BOS-2S, Bentley Laboratories Inc.). The final oxygen capacity of the perfusate at 760 mm Hg was 2.4 vol% (1.1 m*M* O_2). The oxygen tension of perfusate entering the left atrium was from 490 to 520 mm Hg. Gases in the perfusion fluid (partial pressure of carbon dioxide [P_{CO_2}] and oxygen [P_{O_2}] and pH) were determined for each control and before and after injection of vasopressin with a radiometer (Radiometer, ABL2, Acid Base Laboratory, Copenhagen, Denmark).

Determination of myocardial oxygen consumption and cardiac function. Myocardial oxygen consumption ($M\dot{V}O_2$ ml·min^{-1}·g^{-1}) was calculated using the formula:

$$M\dot{V}O_2 = \frac{K \times CF\ (P_{O_{2a}} - P_{O_{2cs}})}{\text{Heart wet weight (g)}},$$

where $P_{O_{2a}}$ and $P_{O_{2cs}}$ are the oxygen partial pressures in millimeters of mercury in the arterial inflow and coronary sinus outflow, respectively; K is the oxygen solubility constant of the perfusion fluid (0.31×10^{-4} ml O_2/ml of KH per mm Hg) and CF is the coronary flow (ml·min^{-1}). Left ventricular end-systolic and end-diastolic pressures, the maximal rate of ventricular pressure change (dP/dt_{max}), coronary flow and cardiac output were determined as described previously (14). After simultaneous collection of afferent left atrial inflow and coronary sinus flow (pulmonary artery outflow), oxygen tension was measured.

Total coronary resistance (mm Hg·ml^{-1}·min^{-1}) was calculated from mean aortic pressure (mm Hg) and coronary flow (ml·min^{-1}). Measurement of large coronary vessel resistance (obtuse marginal artery) was calculated using Poinseuille's equation (15):

$$\text{Large vessel resistance (R)} = \frac{8\mu}{\pi(r_i)^4}.$$

Because π and μ are constants, the formula can be written:

$$R = \left(\frac{1}{r_i}\right)^4,$$

using r_i (internal radius) = d_i (internal diameter)/2; therefore:

$$R = \left(\frac{2}{d_i}\right)^4 (mm^{-4}).$$

The heart rate was maintained at 180 beats/min by pacing the right atrium with a Grass SD9 Stimulator (180 frequency, 7 V, 0.2 ms duration, Grass Instrument Company). Mean aortic pressure was adjusted to between 54 and 58 mm Hg by increasing the outflow resistance. In a number of experiments, the absence of endothelium was verified at the end of the experiment by light microscopy after fixation and staining with hematoxylin-eosin.

Application of vasopressin. The preparation was equilibrated for 20 minutes. Four sets of experiments were carried out. In the first, vasopressin (0.1 U) was injected four times into the tube leading to the left atrium at intervals of 12 minutes. In the second set, the effect of vasopressin in the presence of 8-bromo-cGMP (10^{-4} *M* in 500 ml of perfustate) was studied; 8-bromo-cGMP (10^{-2} *M*) in 5 ml of Krebs-Henseleit solution was added to the perfusate after an equilibration period. Vasopressin was injected four times

JACC Vol. 8, No. 2
August 1986:342–8

at intervals of 12 minutes. The perfusate containing vasopressin as bypassed to avoid contamination of the perfusate. The bypassed volume was replaced with an equal volume of Krebs-Henseleit solution.

In the third set of experiments, the dose-response curves were established between vasopressin and total coronary flow and between vasopressin and total coronary vascular resistance. The dose of vasopressin injected into the left atrium ranged from 0.003 to 0.3 U. The interval between injections depended on the attainment of a steady state. In the fourth set of experiments, the dose-response curves were determined in the presence of 10^{-4} *M* 8-bromo-cGMP, which was added to the perfusate at the onset of the experiment. The dose of vasopressin injected into the left atrium (1 ml over a period of 15 seconds) varied from 0.03 to 1.0 U.

The internal diameter of the obtuse marginal coronary artery was determined with color arteriography during the first two injections of vasopressin (4,16). As described previously, this consisted of gated photography used to determine the internal arterial diameter after injection of 0.5 ml of 0.1% patent blue dye (Sigma Chemical Co.) into the left atrium. The photographic slides were then projected onto a screen with a magnification of 10 and the degree of constriction was quantitated. The resolution (the smallest difference in measurable change in internal diameter) is 0.08 mm (10,13).

Statistical analysis. The Student *t* test for paired and unpaired data was used. Data were expressed as mean ± SEM. Values for the concentration required to obtain 50% of the maximal effect (ED_{50}) in dose-response curves were determined according to the method of Patil et al. (17) and Fleming et al. (18).

Drugs. The following pharmacologic agents were used: 8-bromo-cyclic guanosine 3′:5′-monophosphate (8-bromo-cGMP) (Sigma Chemical Co.) and 8-arginine vasopressin (Parke-Davis).

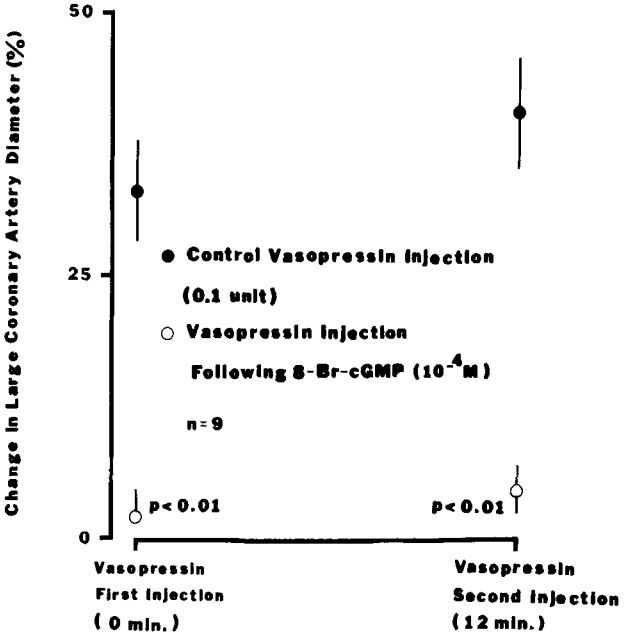
Results

Effect of vasopressin and 8-bromo-cGMP on cardiac performance, pH and myocardial oxygen consumption. The effect of four repeated intraatrial injections of vasopressin (0.1 U) was studied in nine preparations. A significant decline in dP/dt (230 mm Hg·s^{-1}, $p < 0.05$), left ventricular end-systolic pressure (9.5 mm Hg, $p < 0.01$), mean aortic pressure (3.4 mm Hg, $p < 0.05$) and cardiac output (41 ml·min^{-1}, $p < 0.001$) was observed. Left ventricular end-diastolic pressure rose significantly (6.15 mm Hg, $p < 0.01$). Addition of 8-bromo-cGMP eliminated the response to vasopressin. In most instances, the difference between values in the control period and after vasopressin was not significant (dP/dt 118 mm Hg·s^{-1}, left ventricular end-diastolic pressure 0.8 mm Hg; mean aortic pressure 1 mm Hg). Although significant differences were still present between cardiac output and left ventricular end-systolic pressure during the control period and after vasopressin injection (cardiac ouput 9.8 ml·min^{-1}, $p < 0.05$ and left ventricular end-systolic pressure 4.2 mm Hg, $p < 0.01$), these differences were less than before addition of 8-bromo-cGMP ($p < 0.001$).

Changes in dissolved gases and pH in the perfusate after injection of 0.1 U vasopressin were also studied. P_{CO_2} in coronary sinus outflow increased significantly in all experiments (11.1 mm Hg, $p < 0.01$). Because P_{CO_2} in left atrial inflow was constant (31.6 ± 0.2 mm Hg), coronary arteriovenous carbon dioxide difference increased after vasopressin, indicating myocardial ischemia. Ischemia was also responsible for the decline in pH in the coronary outflow (0.1, $p < 0.001$). Myocardial oxygen consumption decreased significantly (0.029 ml·g^{-1}·min^{-1}, $p < 0.001$). After the addition of 8-bromo-cGMP to the perfusate (10^{-4} *M*), no significant differences in P_{CO_2} and pH after vasopressin injection were detected in coronary outflow. Myocardial oxygen consumption also remained unchanged (before 8-bromo-cGMP: 0.061; after 8-bromo-cGMP: 0.058 ml·g^{-1}·min^{-1}).

Effect of vasopressin and 8-bromo-cGMP on large coronary artery diameter and coronary vascular resistance. The injection of vasopressin reduced the internal diameter of the obtuse marginal coronary artery by 36%

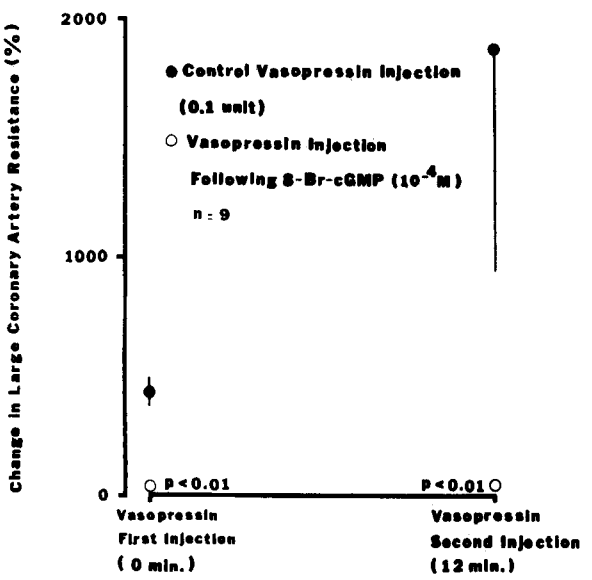
Figure 1. The effect of vasopressin on the internal diameter of the obtuse marginal coronary artery expressed as percentile change. Injections of vasopressin caused a marked diminution in large coronary artery diameter. In the presence of 8-bromo-guanosine 3′:5′-monophosphate (8-Br-cGMP), the diameter of the artery showed little change. The differences are highly significant.



after the first injection and by 40% after the second injection ($p < 0.01$, Fig. 1). After addition of 8-bromo-cGMP, vasopressin failed to constrict the artery (2% after the first and 4.5% after the second injection) (Fig. 1). The difference in response between vasopressin alone and after addition of 8-bromo-cGMP was highly significant ($p < 0.01$ after both injections). As the internal diameter declined, calculated large coronary vascular resistance rose by 1,150% (Fig. 2); 8-bromo-cGMP prevented the increase in large coronary vascular resistance. The differences in response to vasopressin in the absence and presence of 8-bromo-cGMP were highly significant ($p < 0.01$). As a result of vasoconstriction, vasopressin markedly decreased coronary flow (51%) and increased total coronary vascular resistance (120%) (Fig. 3 and 4). Tachyphylaxis to vasopressin was absent, because the response of the coronary flow and resistance remained constant for the period of observation (40 minutes). The presence of 8-bromo-cGMP greatly diminished the fall in coronary flow and the rise in total coronary vascular resistance.

Dose-response curves with vasopressin with and without 8-bromo-cGMP. The dose-response relation between the amount of vasopressin injected and coronary flow is shown in Figure 5; 8-bromo-cGMP significantly inhibited the vasoconstrictor activity of vasopressin in a dose-dependent fashion. There was no evidence of tachyphylaxis. ED_{50} was 0.05 U of vasopressin without and 0.12 U of vasopressin with 8-bromo-cGMP. The dose-response relation between intraatrial vasopressin and total coronary vascular resistance was similar (Fig. 6), with highly significant inhibition of vasoconstriction. ED_{50} was 0.15 U of vasopressin

Figure 2. Vasopressin resulted in a marked percentile increase in large coronary vessel resistance. Large coronary vascular resistance remained unaffected in the presence of 8-bromo-guanosine 3′:5′-monophosphate (8-Br-cGMP). The differences are highly significant.



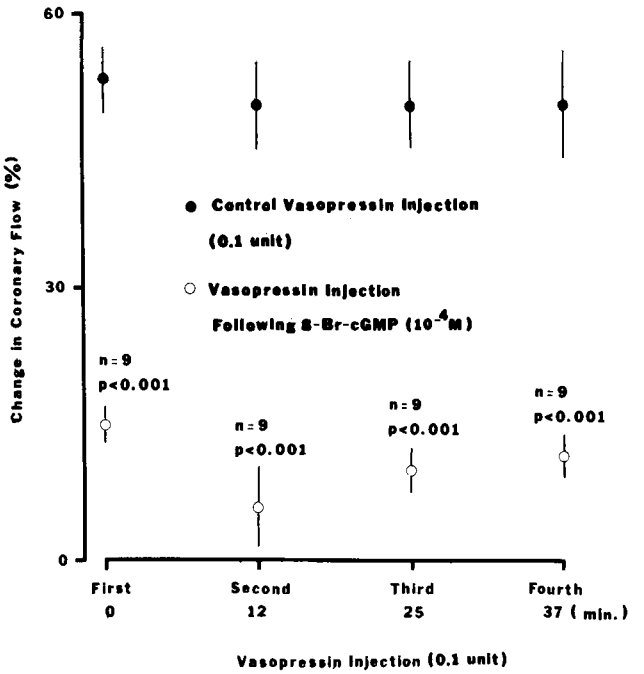
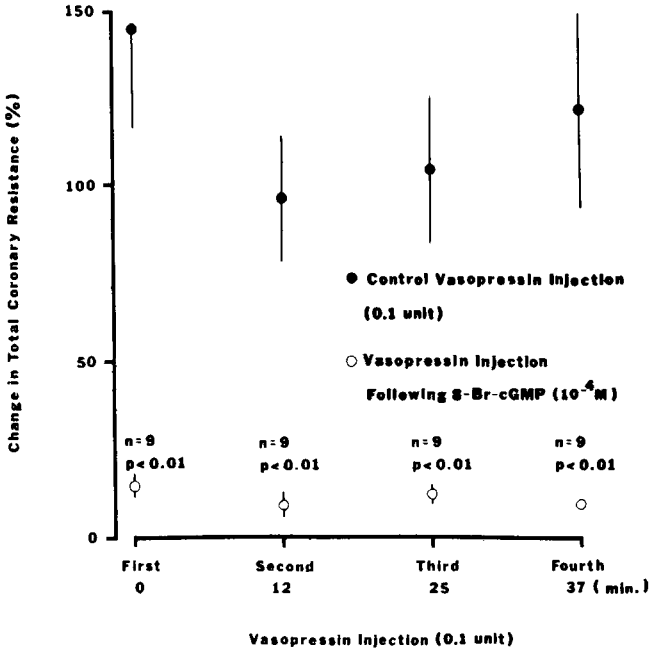


Figure 3. Percentile effect of vasopressin on coronary flow with and without 8-bromo-guanosine 3′:5′-monophosphate (8-Br-cGMP). Vasopressin markedly decreased coronary flow. In the presence of 8-bromo-guanosine 3′:5′-monophosphate vasopressin had little effect. The differences are highly significant.

Figure 4. Effect of vasopressin on percentile changes in total coronary vascular resistance in the presence and absence of 8-bromo-guanosine 3′:5′-monophosphate (8-Br-cGMP). A marked increase of total coronary vascular resistance occurred after injection of vasopressin. In the presence of 8-bromo-guanosine 3′:5′-monophosphate, the increase in total coronary vascular resistance was negligible. The differences are highly significant.



JACC Vol. 8, No. 2
August 1986:342–8

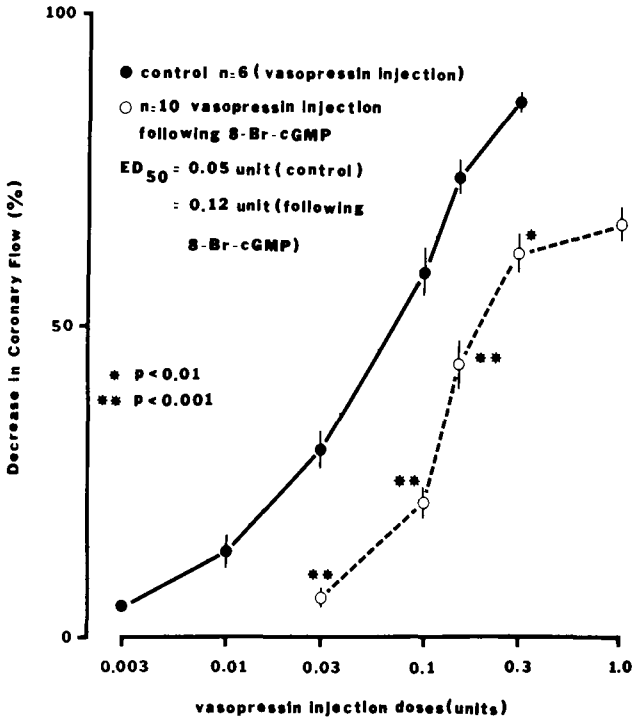


Figure 5. Dose-response relation between vasopressin and total coronary flow. The curve was shifted to the right with the addition of 8-bromo-guanosine 3′:5′-monophosphate (8-Br-cGMP), indicating marked inhibition of vasopressin activity in a dose-dependent fashion and in a noncompetitive manner. ED_{50} was 0.05 U without and 0.12 U in the presence of 8-bromo-guanosine 3′:5′-monophosphate.

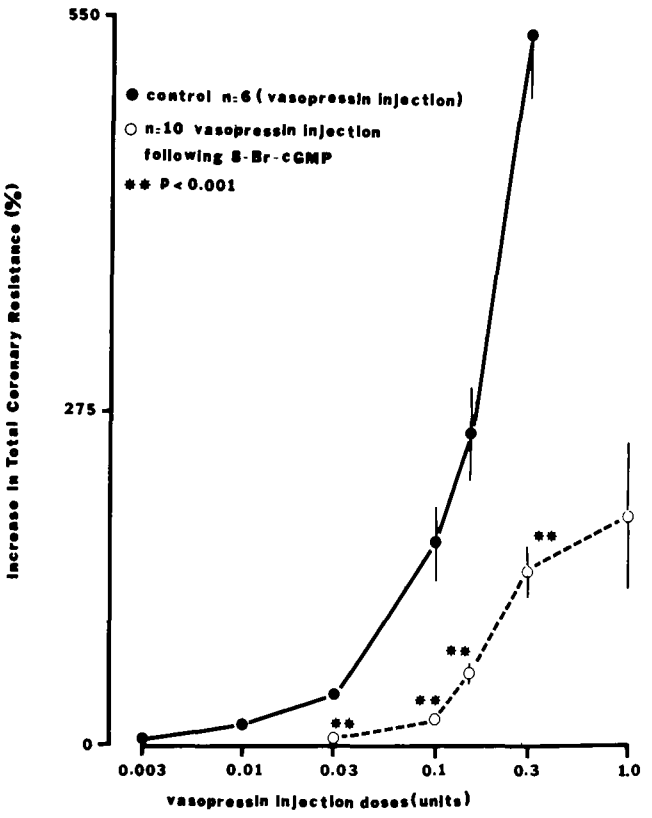


Figure 6. The dose-response relation between vasopressin and total coronary vascular resistance in the absence and presence of 8-bromo-guanosine 3′:5′-monophosphate (8-Br-cGMP). Significant inhibition of coronary vasoconstriction is indicated by a shift of the curve to the right. ED_{50} was 0.15 U without and 0.2 U in the presence of 8-bromo-guanosine 3′:5′-monophosphate.

without and 0.2 U of vasopressin in the presence of 8-bromo-cGMP. The latter inhibited the effect of vasopressin in a noncompetitive manner.

The effect of 8-bromo-cGMP also altered the dose-response curve of left ventricular end-systolic pressure, cardiac ouput, myocardial oxygen consumption, P_{CO_2} and pH to rising doses of vasopressin. As a consequence, the ED_{50} was shifted to the right after doses of vasopressin of from 0.03 to 1 U in the presence of 8-bromo-cGMP (10^{-4} *M*); ED_{50} changed from 0.10 to 0.17 U for left ventricular end-systolic pressure, from 0.07 to 0.14 U for cardiac output and from 0.06 to 0.14 U for myocardial oxygen consumption. The presence of 8-bromo-cGMP also diminished the arteriovenous carbon dioxide difference at high concentrations of vasopressin (for example, at 0.3 U of vasopressin, the coronary arteriovenous P_{CO_2} difference was 50%; 8-bromo-cGMP reduced this difference to 21%). The difference in pH at 0.3 U of vasopressin between coronary arterial and venous outflow was 4%; 8-bromo-cGMP reduced it to 1.5%.

Discussion

Properties of 8-bromo-cGMP and its relation to endothelium. The effect of several vasodilators, such as nitroprusside and nitroglycerin, on vascular smooth muscle is mediated through activation of guanylate cylase (cGMP) (2,6,19). Rapoport and Murad (20) demonstrated that the relaxing effect initiated by endothelium that is responsible for vasodilation (the endothelial-derived relaxing factor of Furchgott and Jothianadan [3]) is also mediated through the formation of cGMP.

An 8-substituted guanosine derivative, 8-bromo-cGMP, has considerably greater vasodilator properties than its unsubstituted parent substance. It was synthesized by Michal et al. (11) in 1970, who examined the effect of various phosphodiesterases on the splitting action of cyclophosphates. They found that the rate of degradation is less when substitution takes place in the 8 position of the purine ring. The cell wall is more readily penetrated by 8-bromo-cGMP than by cGMP, and the former is an effective relaxant of tracheal smooth muscle (11). Like nitroglycerin, 8-bromo-cGMP is also an effective antagonist of vasconstriction initiated by $alpha_2$ receptors (21), which are the dominant receptors in the coronary arteries (22); 8-bromo-cGMP was a more active relaxant. The extent of relaxation depends on the initial tension induced by noradrenaline as the preconstricting agent, as shown by Schultz et al. (23). These findings were confirmed by Saeed and Bing (unpublished data,

1986), who, in addition, discovered that in rabbit aortic rings the relaxing effect was potentiated in arteries deprived of endothelium. Removal of endothelium potentiates the response to other vasoconstrictors as well. For example, Carrier and White (24) found that removal of the vascular endothelium increases the maximal response to selective alpha$_1$ agonists, and Godfraind (25) noted that selective removal of endothelial cells shifted concentration-effect curves of noradrenaline to the left. Like nitroprusside or cGMP, the action of 8-bromo-cGMP is mediated through incorporation of phosphorus-32 into various soluble or particulate proteins in an aortic preparation (4,5).

Antagonistic effects of 8-bromo-cGMP on vasopressin. Our results confirm that 8-bromo-cGMP is effective in dilating constricted coronary arteries that are deprived of endothelium and constricted with vasopressin in perfused supported heart preparations. In this series, vasopressin is used to constrict the vessels; Heyndrickx et al. (26) showed that in doses of 1.2 μmol/kg, vasopressin causes marked coronary constriction (140%). After removal of endothelium, vasopressin induces further constriction in coronary artery strips (27). Our findings demonstrate that vasopressin significantly diminishes large coronary artery diameter (Fig. 1) and increases large coronary artery vascular resistance in the absence of endothelium (Fig. 2). Several workers (27–29) found that in coronary arteries with intact endothelium, vasopressin causes relaxation. In agreement with a series of researchers (26,30–33), we found that the compound also increases total coronary vascular resistance and diminishes coronary flow (Fig. 4 and 5). Coronary flow is also reduced by vasopressin in the study of Wilson et al. (34). Vasopressin causes marked depression of myocardial performance: dP/dt, left ventricular systolic pressure, mean aortic pressure and cardiac output diminish significantly, whereas left ventricular end-diastolic pressure increases. The significant decline in myocardial oxygen consumption, the increase in arteriovenous oxygen difference and in P_{CO_2} of coronary sinus outflow, together with the fall in coronary sinus pH, are indications of myocardial ischemia due to vasospasm. A decrease in myocardial contractility has also been described by Green et al. (35), occurring as a result of myocardial ischemia after sufficiently large doses of vasopressin (3,4,36).

The presence of 8-bromo-cGMP strongly inhibits the vasoconstrictor action of vasopressin; it counteracts the decrease in internal diameter of large coronary vessels as well as the increase in large vessel and total coronary flow (Fig. 4). As a result, myocardial ischemia is absent. In the presence of 8-bromo-cGMP, vasopressin causes no significant changes in pH or P_{CO_2} of coronary sinus effluent and prevents the fall in myocardial oxygen consumption and myocardial contractility. It antagonizes the effects of vasopressin on left ventricular end-diastolic pressure and mean aortic pressure. Although vasopressin still causes significant differences in cardiac output and left ventricular end-systolic pressure, this difference is significantly less than that before the addition of 8-bromo-cGMP.

Influence of 8-bromo-cGMP on dose-response curves of vasopressin. Even more striking is the change in the dose-response relation between intraatrial vasopressin and coronary flow (Fig. 5). The curve for 8-bromo-cGMP is significantly shifted to the right and ED_{50} is 0.05 U before addition of 8-bromo-cGMP and 0.12 U in the presence of 8-bromo-cGMP. The relation of vasopressin to total coronary vascular resistance follows a similar pattern (ED_{50} 0.15 versus 0.2 U) (Fig. 6). This inhibition occurs in a noncompetitive manner. The presence of 8-bromo-cGMP in the perfusate also prevents the decrease in cardiac function, oxygen consumption and development of myocardial acidosis. Apparently, 8-bromo-cGMP is able to overcome the myocardial effects of intense vasoconstriction induced by vasopressin.

Conclusions. Our results regarding coronary arteries perfusing an intact supported heart preparation suggest that 8-bromo-cGMP can be useful in counteracting coronary artery constriction in vivo, particularly when the coronary endothelium has been damaged. We have already shown that the technique described in this report for determination of resistance in large coronary arteries (color arteriography) is applicable to the open chest animal and may lend itself to an investigation of the effect of 8-bromo-cGMP on subepicardial coronary arteries of other large animals and humans.

References

1. Murad F, Arnold WP, Mittal CK, Baughler JM. Properties and regulation of guanylate cyclase and some proposed functions of cyclic GMP. Adv Cyclic Nucleotide Res 1979;11:175–204.
2. Katsuki S, Murad F. Regulation of adenosine cyclic 3′,5′-monophosphate and guanosine cyclic 3′,5′-monophosphate levels and contractility in bovine tracheal smooth muscle. Mol Pharmacol 1977;13:330–41.
3. Furchgott RF, Jothianadan D. Relation of cyclic GMP levels to endothelium-dependent relaxation by acetylcholine in rabbit aorta (abstr). Fed Proc 1983;42:619.
4. Rapoport RM, Draznin MB, Murad F. Sodium nitroprusside-induced protein phosphorylation in intact rat aorta is mimicked by 8-bromo cyclic GMP. Proc Natl Acad Sci USA 1982;79:6470–4.
5. Winquist RJ, Faison EP, Waldman SA, Schwartz K, Murad F, Rapoport RM. Atrial natriuretic factor elicits an endothelium-indpendent relaxation and activates particular guanylate cyclase in vascular smooth muscle. Proc Natl Acad Sci USA 1984;81:7661–4.
6. Schultz K-D, Schultz K, Schultz G. Sodium nitroprusside and other smooth muscle relaxants increase cyclic GMP levels in rat ductus deference. Nature 1977;256:750–1.
7. Holzmann S. Endothelium-induced relaxation by acetylcholine associated with larger rises in cyclic GMP in coronary arterial strips. J Cyclic Nucleotide Protein Phosphor Res 1982;8:409–19.
8. Kukovetz WR, Holzmann S, Poch G. Function of cyclic GMP in acetylcholine-induced contraction of coronary smooth muscle. Naunyn Schmiedebergs Arch Pharmacol 1982;319:29–33.
9. Napoli SA, Gruetter CA, Ignarro LJ, Kadowitz PJ. Relaxation of

bovine coronary arterial smooth muscle by cyclic GMP, cyclic AMP and analogs. J Pharmacol Exp Ther 1980;212:469–73.

10. Saeed M, Schmidli J, Metz M, Bing RJ. Perfused rabbit heart: endothelium-derived relaxing factor in coronary arteries. J Cardiovasc Pharmacol 1986:8:257–61.
11. Michal G, Nelboeck M, Weimann G. Cyclophosphates III-Spaltung verschiedener Cyclophosphate durch Phosphodiesterase aus Herz und Fettgewebe. Z Anal Chem 1970;252:189–93.
12. Kukovetz WR, Holzmann S, Wurm A, Poch G. Evidence for cyclic GMP-mediated relaxant effects of nitrocompounds in coronary smooth muscle. Naunyn Schmiedebergs Arch Pharmacol 1979;310:129–38.
13. Bing RJ, Burger W, Chemnitius JM, Saeed M, Metz M. Effects of intact endothelium against platelet-induced coronary artery spasm in isolated rabbit hearts. Am J Cardiol 1985;55:1596–600.
14. Chemnitius JM, Burger W, Bing RJ. Crystalloid and perfluorochemical perfusates in an isolated working rabbit heart preparation. Am J Physiol 1985;249:H285–92.
15. Vatner SF, Pagani M, Manders WT, Pasipoularides AD. Alpha adrenergic vasoconstriction of large coronary arteries in the conscious dog. J Clin Invest 1980;65:5–14.
16. Burger W, Chemnitius JM, Bing RJ. A new method for visualization of subepicardial coronary arteries in small isolated mammalian hearts. Proc Soc Exp Biol Med 1985;178:309–12.
17. Patil PN, Fudge K, Jacobowitz D. Steric aspects of adrenergic drugs. XVIII. α-Adrenergic receptors of mammalian aorta. Eur J Pharmacol 1972;19:79–87.
18. Fleming WW, Westfall EP, De La Lande IS, Jellet LB. Long-normal distribution of equieffective doses of norephinephrine and acetylcholine in several tissues. J Pharmacol Exp Ther 1972;181:339–45.
19. Katsuki S, Arnold W, Mittal C, Murad F. Stimulation of guanylate cyclase by sodium nitroprusside, nitroglycerin and nitric oxide in various tissue preparations and comparison to the effects of sodium azide and hydroxylamine. J Cyclic Nucleotide Res 1977;3:23–5.
20. Rapoport RM, Murad F. Agonist-induced endothelium-dependent relaxation in rat thoracic aorta may be mediated through cGMP. Circ Res 1983;52:352–7.
21. Eskinder H, Gross GJ. Selective inhibition of alpha$_2$ (α_2) versus alpha$_1$ (α_1)-mediated vasoconstrictor responses by nitroglycerin (GTN) and 8-bromo cyclic GMP (8-Br-cGMP) in canine saphenous vein (abstr). Circulation 1985;2(suppl II):II-51.
22. Saeed M, Holtz J, Elsner D, Bassenge E. Sympathetic control of myocardial oxygen balance in dogs mediated by activation of coronary vascular α_2-adrenoceptors. J Cardiovasc Pharmacol 1985;7:167–73.
23. Schultz K-D, Bohme E, Kreye VAW, Schultz G. Relaxation of hormonally stimulated smooth muscular tissues by the 8-bromo derivative of cyclic GMP. Naunyn Schmiedebergs Arch Pharmacol 1979;306:1–9.
24. Carrier GO, White RE. Enhancement of alpha-1 and alpha-2 adrenergic agonist-induced vasoconstriction by removal of endothelium in rat aorta. J Pharmacol Exp Ther 1985;232:682–7.
25. Godfraind T. Calcium entry and calcium entry blockade. In: Godfraind T, Vanhoutte PM, Govoni S, Paoletti R, eds. Calcium Entry Blockers and Tissue Protection. New York: Raven, 1985:1–19.
26. Heyndrickx GR, Boettcher EH, Vatner SF. Effects of angiotensin, vasopressin, and methoxamine on cardiac function and blood flow distribution in conscious dogs. Am J Physiol 1976;231:1579–87.
27. Katusic ZS, Shepherd JT, Vanhoutte PM. Vasopressin causes endothelium-dependent relaxations of the canine basilar artery. Circ Res 1984;55:575–9.
28. Turlapaty PDMV, Altura BM. Effects of neurohypophyseal peptide hormones on isolated coronary arteries: role of magnesium ions. Exp Clin Res 1982;1:122–8.
29. Altura BM, Altura BT. Actions of vasopressin, oxytocin and synthetic analogs on vascular smooth muscle. Magnesium. Fed Proc 1984;43:80–6.
30. Pantely GA, Ladley HD, Anselone CG, Bristow JD. Vasopressin-induced coronary constriction at low perfusion pressures. Cardiovasc Res 1985;19:433–41.
31. Arnim TV, Crea F, Chierchia S, Thompson GR, Maseri A. Effects of vasoactive stimuli on coronary vascular resistance in isolated perfused rabbit hearts: no vasospastic response to ergonovine with and without atherogenic diet. Basic Res Cardiol 1983;78:415–22.
32. Khayyal MA, Eng C, Franzen D, Breall JA, Kirk ES. Effects of vasopressin on the coronary circulation: reserve and regulation during ischemia. Am J Physiol 1985;248:H516–22.
33. Kopia GA, Valocik RE. Antagonism of vasopressin-induced coronary artery constriction by the vasopressin antagonist d $(CH_2)_5$ Tyr (Me)-AVP. J Cardiovasc Pharmacol 1985;7:958–63.
34. Wilson MF, Brackett DJ, Archer LT, Hinshaw LB. Mechanisms of impaired cardiac function by vasopressin . Ann Surg 1980;191:494–500.
35. Green HD, Wegria R, Boyer NH. Effects of epinephrine and pitressin on the corronary artery inflow in anesthetized dogs. J Pharmacol Exp Ther 1942;76:378–91.
36. Nakano J. Studies on the cardiovascular effect of synthetic vasopressin. J Pharmacol Exp Ther 1967;157:19–31.