The Effects of $\alpha_2$-Adrenergic and Serotonergic Receptor Antagonists on Cyclic Blood Flow Alterations in Stenosed Canine Coronary Arteries


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SUMMARY. Platelets possess $\alpha_2$-adrenergic and serotonergic (5-hydroxytryptamine) receptors which are thought to mediate the in vitro proaggregatory effects of epinephrine and serotonin, respectively. However, their importance in platelet aggregation in vivo is uncertain. In the present study, we evaluate the ability of yohimbine and ketanserin, relatively selective $\alpha_2$-adrenergic and serotonin antagonists, respectively, to alter cyclic flow reductions in stenosed coronary arteries in open-chest, anesthetized dogs. These cyclic flow reductions, characterized by progressive declines in coronary blood flow interrupted by abrupt and, often spontaneous, restorations of flow, were produced by cylindrical constrictors placed on the left anterior descending coronary artery. A pulsed Doppler flow probe, placed proximal to the constrictor, was used to measure coronary blood flow. Regional myocardial blood flow was measured with 15-μm radiolabeled microspheres before coronary constriction and when coronary blood flow appeared to be at its nadir and zenith during cyclic flow reductions. After the cyclic flow reductions had been observed for 1 hour, yohimbine (1-2 mg/kg), ketanserin (0.25 or 0.5 mg/kg), or saline was given, and coronary blood flow and hemodynamics were monitored for another hour. The frequency of cyclic flow reductions and the mean of the three lowest nadirs of coronary blood flow (mean ± SE) were compared between the first and second hours. Ketanserin, at doses of 0.25 and 0.50 mg/kg, virtually abolished cyclic flow reductions in all dogs tested. Yohimbine [1 mg/kg ($n = 14$)] was partially effective in reducing the frequency (9.6 vs. 5.5 cyclic flow reductions/hr) and severity of cyclic flow reductions (nadirs of coronary blood flow = 6.2 ± 2.4 vs. 20.9 ± 6.1% of control). A higher dose of yohimbine [2 mg/kg ($n = 7$)] was no more effective. The frequency (9.3 ± 0.9 vs. 9.3 ± 1.0 CFR/hr) and severity (17.4 ± 5.4 vs. 12.4 ± 3.9% of control coronary blood flow) of cyclic flow reductions were not changed by saline. The relatively selective $\alpha_1$-adrenergic antagonist, prazosin (0.01 mg/kg, iv), and the $\beta$-adrenergic antagonist, propranolol (1-2 mg/kg, iv), did not affect the frequency or severity of cyclic flow reductions. Thus, the abilities of yohimbine to inhibit and ketanserin to abolish cyclic flow reductions in stenosed canine coronary arteries suggest that serotonin and, possibly, $\alpha_2$-adrenergic agonists may influence cyclic flow alterations importantly in this model. (Circ Res 55: 642-652, 1984)
anesthetized dogs. To confirm that adrenergic stimulation of platelet aggregation in vivo is mediated via α₂-stimulation, we compared the effects of yohimbine to those of prazosin, a relatively pure α₁-adrenergic antagonist (Brogden et al., 1977) and to those of propranolol, a nonselective β-adrenergic antagonist.

Ketanserin, a new, relatively selective 5HT₂ receptor-antagonist, antagonizes the actions of serotonin in a variety of experimental preparations (Janssen, 1983; Vanhoutte, 1983). In vitro, ketanserin has been shown to compete for 5HT₂ binding sites on platelets and to inhibit their aggregation induced by exogenously added 5HT (Vanhoutte, 1983). The suggestion that ketanserin also possesses α₁-adrenergic blocking properties provided further impetus to evaluate the relatively selective α₁-adrenergic antagonist, prazosin, in the same model. Thus, these studies were designed to evaluate the roles of potentially important mediators of in vivo platelet aggregation in a well-defined model of coronary stenosis.

Methods

Surgical Preparation

Mongrel dogs (17–27 kg) of either sex were anesthetized with sodium pentobarbital (30 mg/kg, iv), and ventilated artificially with room air, delivered by a Harvard respirator. Tidal volume and respiratory rate were adjusted to maintain blood gases and pH within physiological limits. Aortic and venous catheters were inserted via the common carotid artery and external jugular vein, respectively. Heating pads were placed under the animal to maintain rectal temperature between 37 and 39°C. After a thoracotomy in the 5th left intercostal space, the heart was suspended in a pericardial cradle. A Konigsberg pressure transducer (P22) was inserted into the left ventricular apex and another catheter into the left atrium for injecting tracer microspheres. A segment of the left anterior descending (LAD) or left circumflex (LCX) coronary artery was dissected away from surrounding tissue, and a flow probe, connected to a 20-MHz pulsed Doppler flow probe [manufactured by Dr. C. J. Hartley (Hartley et al., 1978)] was placed on the coronary artery proximal to where a constriction would subsequently be placed (Fig. 1). Also, a small polyethylene catheter was positioned in the distal end of a diagonal branch of the LAD below the site of constriction, enabling the pressure gradient across the stenosis to be measured.

Experimental Protocol

After instrumentation was completed, dogs were allowed to stabilize for one-half hour. Control hemodynamic measurements, including heart rate, arterial blood pressure, LV dP/dt, as well as pulsatile and mean coronary flow, were recorded continuously on a Hewlett-Packard model (7758) eight-channel recorder. The Doppler flow probes were calibrated on coronary arteries in separate animals, as described previously (Bush et al., 1984). Regional myocardial blood flow (RMBF) was measured with 15-μm carbonized microspheres labeled with 153Sm, 85Sr, 95Nb, or 57Co. Microspheres were sonicated and vortexed for several minutes before 1–2 million beads were injected into the left atrium over an 8 to 10-second period. Starting 10 seconds before and continuing for 90 seconds after microsphere injection, reference arterial blood was withdrawn from the carotid artery at a constant rate of 7.8 ml/min with a Harvard infusion/withdrawal pump. The order of isotopes was changed randomly.

After obtaining control measurements, we measured the hyperemic response following a 10-second total occlusion. Next, a hard plastic, cylindrical constrictor was placed on the coronary artery 3–10 mm distal to the flow probe. Cyclic flow reductions, thought to represent platelet aggregation and release, were produced after identifying a constrictor that eliminated or attenuated the hyperemic response after a 10-second occlusion and reduced resting coronary blood flow by at least 15% (Gallagher et al., 1978; Klocke, 1983). The cyclic coronary flow reductions (CFR) were observed for 1 hour, during which CBF was monitored continuously. RMBF was measured when CBF was approximately at its nadir and immediately after restorations of flow. During the hour of observation, the frequency of CFR and the zenith and nadir of CBF were recorded.

After 1 hour of CFR, yohimbine (1.0–2.0 mg/kg), prazosin (0.01 mg/kg), ketanserin (0.25 or 0.50 mg/kg), propranolol (1.0–2.0 mg/kg), or an equal volume of saline was administered intravenously over 1 minute. The pressor response to phenylephrine (3 μg/kg, iv) was monitored just before, and at least 5 minutes after, prazosin administration. Hemodynamic variables and CFR were monitored for another hour, and the changes in CBF pattern were compared to those occurring during the first hour. To compare the changes in CBF, RMBF, and distal coronary pressure that occurred during nadirs of CBF with those occurring with the most severe degree of ischemia, the constricted coronary artery was totally occluded at the end of each experiment, and all measurements (including RMBF) were repeated within 3 minutes after occlusion. Next, with the coronary artery still totally occluded, 3–5 ml of 10% (wt/vol) alphazurine (patent blue violet) were injected into the distal coronary catheter, followed quickly by electrical fibrillation of the heart. This allowed a delin-
and instrumented with carotid arterial and jugular venous catheters. After a 30-minute stabilization period, during which time arterial blood pressure and heart rate were monitored, venous blood was collected in 1/10 volume of 3.5% sodium citrate (pH 7.4). Yohimbine (2 mg/kg, iv) was administered, and at the end of a 30-minute period, blood was obtained for platelet studies. Platelet-rich plasma was obtained by centrifuging the blood at 75 g for 20 minutes (Campbell et al., 1981). Platelet-poor plasma was prepared by centrifuging the red cell suspension further at 1500 g for 10 minutes. Platelet aggregation was determined in control and yohimbine-treated samples in vitro by the method of Born (1962). Platelet-rich plasma (0.4 ml) was incubated at 37°C with stirring for 1 minute in a Sienco dual channel aggregometer. Serotonin (2 × 10^−6 M) or its vehicle was added, and after 0.5 minute, adenosine diphosphate (ADP) (0.5–10 μM) was added and aggregation was quantified as change in light transmission. The results were expressed as percent of maximal aggregation.

Materials

Sodium pentobarbital (50 mg/ml) was purchased from Abbott Laboratories; alphazurine (patent blue violet), indomethacin, and yohimbine hydrochloride were obtained from Sigma Chemical Company. Prazosin (stock solution 0.5 mg/ml) dissolved in glycerine and propranolol hydrochloride were kindly provided by Pfizer and Ayerst, respectively. Ketanserin (tartrate salt), was generously provided by Dr. J.M. Van Nueten, Janssen Pharmaceutica. 6-Keto PGF_1α and TXB_2 were obtained from the Upjohn Co. The doses of all drugs are of the salt.

Statistical Analyses

All values are expressed as mean ± se. Analysis of variance (ANOVA) was used to determine whether significant differences existed between three or more treatment groups. Duncan’s multiple range or Newman-Keuls tests (Zar, 1974) were used to identify which group mean values differed significantly. Paired Student’s t-test was used to determine whether a significant change in a variable occurred within animals after saline or drug between the first and second hours of observation. In all cases, a P value <0.05 was considered significant.

Results

Baseline Characteristics of the Model

Before production of the CFR, the animals in all groups were similar with regard to basal CBF, maximal hyperemic response, constriction-induced reduction of basal coronary flow, and other hemodynamic variables. Among 61 dogs, CFR were successfully produced in 51 animals. In these 51 animals, 14 received saline; 14 received either 0.25 (six dogs) or 0.50 (eight dogs) mg/kg of ketanserin; 14 received yohimbine; four received propranolol; and five received prazosin. The average degree of reduction of resting CBF by coronary stenoses following coronary constriction was 53 ± 2% for phasic CBF and 40.3 ± 3% for mean flow. As expected, this degree of flow reduction almost completely abolished the hyperemic response following a 10-second

Hemodynamic Effects of Yohimbine and Ketanserin

We measured heart rate, aortic pressure, LV dP/dt max, and CBF before and after administration of either drug in 11 open-chest, anesthetized dogs without coronary constrictions. Yohimbine (n = 7 dogs) was given in cumulative doses of 0.5, 1.0, and 2.0 mg/kg, intravenously at 10-minute intervals, and ketanserin (n = 4 dogs) in cumulative doses of 0.25, 0.50, and 1.0 mg/kg, iv. To determine whether the doses of yohimbine and ketanserin that abolished CFR produced significant α_1-blockade, we monitored the pressor response to 3 μg/kg (iv) phenylephrine (bolus) before and after yohimbine (1.0 mg/kg) and ketanserin administration (0.5 mg/kg).

Assessment of Platelet Aggregation in Vitro

A separate set of six dogs was anesthetized with sodium pentobarbital (30 mg/kg, iv), ventilated with room air, and instrumented with carotid arterial and jugular venous catheters. After a 30-minute stabilization period, during which time arterial blood pressure and heart rate were monitored, venous blood was collected in 1/10 volume of 3.5% sodium citrate (pH 7.4). Yohimbine (2 mg/kg, iv) was administered, and at the end of a 30-minute period, blood was obtained for platelet studies. Platelet-rich plasma was obtained by centrifuging the blood at 75 g for 20 minutes (Campbell et al., 1981). Platelet-poor plasma was prepared by centrifuging the red cell suspension further at 1500 g for 10 minutes. Platelet aggregation was determined in control and yohimbine-treated samples in vitro by the method of Born (1962). Platelet-rich plasma (0.4 ml) was incubated at 37°C with stirring for 1 minute in a Sienco dual channel aggregometer. Serotonin (2 × 10^−6 M) or its vehicle was added, and after 0.5 minute, adenosine diphosphate (ADP) (0.5–10 μM) was added and aggregation was quantified as change in light transmission. The results were expressed as percent of maximal aggregation.

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Mean Cor Flow z- (kHz)

**FIGURE 2.** A representative recording from a dog with a severe coronary stenosis and cyclic flow reductions. Note the declines in distal coronary artery pressure, phasic, and mean CBF during a 10-second total LAD occlusion and during CFR. Ketanserin abolished the CFR in this representative animal.

Subtotal coronary occlusion is known to compromise subepicardial and subendocardial perfusion. Therefore, to evaluate further this experimental model, we used radiolabeled microspheres to examine the changes in transmural blood flow during the nadir and zenith of CBF during the CFR (Table 1). Both subepicardial and subendocardial blood flow in the ischemic region declined significantly during nadirs of CBF. Relative to values during nadirs of CBF, subepicardial blood flow increased during restorations of blood flow, but rarely exceeded or reached control values. Total occlusion of the stenosed coronary artery at the end of each experiment produced greater declines in both subepicardial (0.19 ± 0.05 ml/min per g) and subendocardial (0.12 ± 0.06 ml/min per g) flow. Except for the endocardial layer of the ischemic region in yohimbine-treated dogs, after restoration of coronary blood flow, there were no differences between treatment groups in any other region or layer.

**Effects of Interventions on CFR**

The frequency of CFR was unchanged after 1 hour in dogs that received saline: 9.3 ± 0.9 vs. 9.3 ± 1.0 CFR/hr, reflecting the stability of the experimental model (Fig. 3). Ketanserin, at doses of 0.25 and 0.5 mg/kg, evaluated in separate groups of dogs, virtually abolished CFR (Figs. 2 and 3). There was no difference in the efficacy of these two doses. The frequency of CFR was reduced from 14.0 ± 2.1 to 2.8 ± 1.8 CFR/hr (P < 0.01) in six dogs that received 0.25 mg/kg and from 10.5 ± 2.1 to 0.2 ± 0.2 CFR/hr (P < 0.01) in eight dogs given the higher dose. Since there was no difference in the effect of these two doses of ketanserin on the frequency or severity of cyclic flow reductions, the results from these two subgroups of dogs were combined, as shown in Figure 3. The number of CFR/hr during the second hour in ketanserin-treated dogs was not zero be-
TABLE 1

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Level</th>
<th>n</th>
<th>Control CBF</th>
<th>Nadir of CBF</th>
<th>Restoration of CBF</th>
<th>Total coronary occlusion</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Ischemic region</td>
<td>Nonischemic region</td>
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<tr>
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<td>0.91 ± 0.06</td>
<td>0.32 ± 0.06*</td>
<td>0.90 ± 0.16</td>
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<td></td>
<td>Endo</td>
<td></td>
<td>0.93 ± 0.09</td>
<td>0.22 ± 0.05*</td>
<td>0.63 ± 0.14†</td>
<td>0.12 ± 0.03*</td>
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<td>Ketanserin</td>
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<td>0.96 ± 0.06</td>
<td>0.18 ± 0.04†</td>
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<tr>
<td></td>
<td>Endo</td>
<td></td>
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<td>0.45 ± 0.09*</td>
<td>0.93 ± 0.15</td>
<td>0.12 ± 0.03†</td>
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<td>0.63 ± 0.07</td>
<td>0.21 ± 0.08*</td>
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<tr>
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<td>Endo</td>
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<td>0.81 ± 0.07</td>
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<td>0.43 ± 0.07†</td>
<td>0.12 ± 0.05*</td>
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<td>Epi</td>
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<td>0.29 ± 0.16*</td>
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<tr>
<td></td>
<td>Endo</td>
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<td>1.25 ± 0.04</td>
<td>0.27 ± 0.19*</td>
<td>0.81 ± 0.27†</td>
<td>0.18 ± 0.12*</td>
</tr>
<tr>
<td>Prazosin</td>
<td>Epi</td>
<td>4</td>
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<td>0.35 ± 0.07*</td>
<td>1.28 ± 0.32</td>
<td>0.18 ± 0.04*</td>
</tr>
<tr>
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<td>0.89 ± 0.21</td>
<td>0.20 ± 0.02*</td>
<td>1.02 ± 0.23</td>
<td>0.08 ± 0.02*</td>
</tr>
</tbody>
</table>

Regional myocardial blood flow (RMBF) was measured with 15-μm radiolabeled microspheres before coronary constriction, during approximately the nadirs and zeniths of CBF of the cyclic flow reductions, and after total coronary occlusions at the end of the experiment. Except for the last time point, all other measurements of RMBF were made before any drug or saline was given.

* Significantly different from control, P < 0.05 (ANOVA, Duncan's multiple range test).
† Significantly different from all other time points (P < 0.05, ANOVA, Duncan's multiple range test).
‡ Significantly different (lower) from other treatment groups.

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cause the onset of effect of ketanserin sometimes took several minutes. Yohimbine, evaluated in 14 dogs, was not uniformly effective in abolishing CFR. CFR were abolished in four dogs. The frequency of CFR was reduced significantly by yohimbine at 1 mg/kg from 9.6 ± 1.1 to 5.5 ± 1.1 CFR/hr. The dose of yohimbine increased to 2.0 mg/kg (cumulative) in seven dogs in which the 1 mg/kg dose was ineffective or only partially effective. The 2 mg/kg dose did not significantly alter the frequency of CFR: 9.8 ± 1.5 vs. 7.6 ± 2.0 CFR/hr. The relatively selective α1-adrenergic antagonist, prazosin, (n = 5 dogs) did not affect CFR (7.8 ± 1.5 vs. 8.0 ± 1.5 CFR/hr), nor did the β-adrenergic antagonist, propranolol, in four dogs (1-2 mg/kg; 8.5 ± 1.2 vs. 9.5 ± 1.3 CFR/hr).

Further quantification of the severity of cyclic flow reductions was provided by averaging the three lowest nadirs of coronary blood flow before and after drug intervention. In those cases where cyclic flow reductions were nearly or completely abolished, the three lowest levels of coronary blood flow during the second hour of the protocol were averaged. The severity of reduction in CBF during CFR ("nadir") was constant in saline-treated, control dogs (17.4 ± 5.4 vs. 12.4 ± 3.9% of control CBF; P = NS) (Fig. 3B). By abolishing CFR, ketanserin also maintained CBF at a level significantly higher than during the CFR: 3.0 ± 1.0% (control for both doses) vs. 42.3 ± 9.0 and 56.3 ± 1.9% of control for 0.25 and 0.50 mg/kg ketanserin, respectively. Yohimbine, 1 mg/kg, increased the "nadir" of CBF significantly, from 6.2 ± 2.4 to 20.9 ± 6.1% of control (n = 14). The higher dose (2.0 mg/kg) did not improve CBF further (3.0 ± 12 vs. 13.0 ± 7.3% of control, respectively). Neither prazosin (2.8 ± 2.6 vs 4.4 ±
A. FREQUENCY OF CYCLIC FLOW REDUCTIONS (CFRs)

![Graph showing frequency of cyclic flow reductions per hour before and after saline, ketanserin, or yohimbine administration.](image)

**Figure 3.** Panel A: the number of cyclic flow reductions per hour before ("pre") and after ("posf") saline, ketanserin, or yohimbine administration. Panel B: the maximum depression, or nadir, of coronary blood flow (CBF) during CFR was computed by averaging the three lowest values during each 1-hour interval. In dogs whose CFR were abolished, the three lowest values of CBF (which were recorded at 10-minute intervals) were averaged.

B. SEVERITY (NADIR) OF CORONARY BLOOD FLOW DURING CFR

![Graph showing severity (nadir) of coronary blood flow during CFR.](image)

**Figure 4.** Panel A: changes in aortic and distal coronary arterial TXB\(_2\) before [control (con)] and during CFR, and after ketanserin administration (n = 9 dogs). Blood (plasma) samples were collected and assayed for TXB\(_2\) (stable breakdown product of TXA\(_2\)) as described in text. *Significantly different from control (preconstriction) value only (P < 0.02). Panel B: aortic and distal coronary arterial concentration of 6-keto PGF\(_{1\alpha}\) (stable breakdown product of PGI\(_2\)) at the same time points as in panel A. * Significantly different, compared to control. Panel C: the ratio between 6-keto PGF\(_{1\alpha}\) and TXB\(_2\) concentrations in the aortic and distal coronary artery at the various time periods evaluated are shown. The ratios of PGF\(_{1\alpha}\) and TXB\(_2\) at control, during CFR, and after ketanserin administration are shown.

Hemodynamic Changes during CFR

Table 2 shows the hemodynamic changes that occurred during control conditions after CFR were produced by coronary stenoses, and after drug or saline administration. There were no differences

- Propranolol (8.5 ± 1.2 vs. 9.5 ± 1.3%) altered the severity of CBF reductions.
- We wished to determine whether the efficacy of ketanserin in this model was due to inhibition of thromboxane (TXA\(_2\)) and/or increase in prostacyclin (PGI\(_2\)) generation in the stenosed coronary arteries. Figure 4 shows the concentrations of TXB\(_2\) and 6-keto PGF\(_{1\alpha}\) (stable breakdown products of TXA\(_2\) and PGI\(_2\), respectively) in blood sampled from the aorta and distal coronary catheters before (preconstriction) and after CFR and following ketanserin administration. TXB\(_2\) levels in the distal coronary artery rose significantly during CFR, from a control value of 40 ± 7 to 163 ± 42 pg/ml (Fig. 4A). Abolition of CFR by ketanserin was accompanied by a decline in these levels to 95 ± 29 pg/ml; this value was not significantly different from either the control value or during the CFR before ketanserin administration (Fig. 4A).
- Aortic TXB\(_2\) concentrations also increased during CFR (65 ± 24 to 125 ± 35) and declined after ketanserin was given (Fig. 4A). Concentrations of 6-keto PGF\(_{1\alpha}\) in the distal coronary bed also increased significantly, from 122 ± 24 to 290 ± 61 pg/ml during CFR, and remained elevated after ketanserin administration (305 ± 57) (Fig. 4B).
- Aortic concentrations of 6-keto PGF\(_{1\alpha}\) did not change significantly during CFR or after ketanserin. Figure 4C shows the ratio of 6-keto PGF\(_{1\alpha}\) to TXB\(_2\) concentrations in aortic and distal coronary blood at each of these time points. The aortic 6-keto-PGF\(_{1\alpha}\):TXB\(_2\) ratio (Fig. 4C) declined slightly from 4.4 ± 1.9 to 2.1 ± 0.6 during CFR, and increased to 3.4 ± 0.8 after ketanserin administration. This ratio in the distal coronary artery also fell insignificantly during CFR, and did not increase significantly after ketanserin administration.
between any of the treatment groups in any hemodynamic variable before and during the first hour of CFR. Saline-treated control dogs did not display any significant hemodynamic changes throughout the remaining experimental protocol. Propranolol lowered heart rate and LV dp/dt significantly, whereas yohimbine, ketanserin, and prazosin all lowered aortic pressure. The pressure response to a single iv bolus injection of phenylephrine (3 μg/kg) was blunted by the lowest dose of prazosin utilized in this study (change in systolic pressure/change in diastolic pressure): 40 ± 8/43 ± 17 before prazosin vs. 8 ± 4/9 ± 4 mm Hg (P < 0.02) (n = 3 dogs) after prazosin.

### Hemodynamic Effects of Ketanserin and Yohimbine in Dogs without Coronary Stenoses

Table 3 shows the hemodynamic effects of ketanserin and yohimbine, when evaluated in an additional 11 dogs without coronary arterial stenoses. Yohimbine lowered aortic pressure significantly and dose dependently. It also caused significant and dose-dependent declines in heart rate and coronary blood flow. Ketanserin also lowered aortic pressure significantly and dose dependently.

### In Vitro Assessment of Platelet Aggregation

Adenosine diphosphate produced a concentration-related increase in platelet aggregation (Fig. 5). When added to platelet-rich plasma, serotonin (10^{-6} to 10^{-5} m) did not alter aggregation. However, platelets incubated with serotonin (2 × 10^{-6} m) exhibited an enhanced response to ADP, as evidenced by the shift to the left of the ADP concentration-response curve. Similar effects were observed in both pre- and post-yohimbine-treated samples, suggesting that, at the doses studied, yohimbine does not exert any significant serotonergic blocking effect. Epinephrine (10^{-6} m)-induced platelet aggregation was also potentiated by serotonin (2 × 10^{-6} m) in control (76 ± 4.5% of maximal aggregation occurring with ADP stimulation, n = 4) but not in yohimbine-treated dogs (0 ± 0%, n = 6). These data emphasize the adrenergic blocking effect of yohimbine administered in vivo on platelet aggregation evaluated in vitro.

### Discussion

In this experimental model, a severe coronary arterial constriction sufficient to reduce basal CBF and abolish the hyperemic response following a 10-second total occlusion usually results in a cyclic pattern of flow suggestive of alternating platelet aggregation, and subsequent dislodgement. Angiographic, histological, and pharmacological studies from other laboratories (Folts et al., 1976, 1982), and our own (Bush et al., 1984), strongly suggest that these CFR are caused primarily by platelet, red
TABLE 3

Hemodynamic Effects of Ketanserin and Yohimbine in Dogs without Coronary Constrictions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heart rate (beats/min)</th>
<th>AOS (mm Hg)</th>
<th>AOD (mm Hg)</th>
<th>LV dP/dt</th>
<th>CBF (kHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>1 min</td>
<td>10 min</td>
<td>1 min</td>
<td>10 min</td>
</tr>
<tr>
<td>Ketanserin</td>
<td>Yohimbine</td>
<td>7</td>
<td>145</td>
<td>131</td>
<td>129</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±13</td>
<td>±11*</td>
<td>±12*</td>
<td>±9*</td>
</tr>
<tr>
<td>Ketanserin</td>
<td>Yohimbine</td>
<td>4</td>
<td>162</td>
<td>162</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±13</td>
<td>±16</td>
<td>±16</td>
<td>±18</td>
</tr>
<tr>
<td>Ketanserin</td>
<td>Yohimbine</td>
<td>7</td>
<td>127</td>
<td>119</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±11</td>
<td>±7</td>
<td>±8</td>
<td>±10*</td>
</tr>
<tr>
<td>Ketanserin</td>
<td>Yohimbine</td>
<td>4</td>
<td>124</td>
<td>104</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±3</td>
<td>±3*</td>
<td>±3*</td>
<td>±2†</td>
</tr>
<tr>
<td>Ketanserin</td>
<td>Yohimbine</td>
<td>7</td>
<td>104</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±10</td>
<td>±8</td>
<td>±8</td>
<td>±10*</td>
</tr>
<tr>
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<td>Yohimbine</td>
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<td>102</td>
<td>80</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±1</td>
<td>±3*</td>
<td>±2*</td>
<td>±7*</td>
</tr>
<tr>
<td>Ketanserin</td>
<td>Yohimbine</td>
<td>7</td>
<td>1880</td>
<td>1605</td>
<td>1671</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±161</td>
<td>±61</td>
<td>±121</td>
<td>±83</td>
</tr>
<tr>
<td>Ketanserin</td>
<td>Yohimbine</td>
<td>4</td>
<td>1863</td>
<td>1849</td>
<td>1746</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±136</td>
<td>±194</td>
<td>±230</td>
<td>±170</td>
</tr>
<tr>
<td>Ketanserin</td>
<td>Yohimbine</td>
<td>7</td>
<td>1.76</td>
<td>1.57</td>
<td>1.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.23</td>
<td>±0.21</td>
<td>±0.25*</td>
<td>±0.20*</td>
</tr>
<tr>
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<td>Yohimbine</td>
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<td>1.47</td>
<td>1.27</td>
<td>1.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.27</td>
<td>±0.18</td>
<td>±0.24</td>
<td>±0.23</td>
</tr>
</tbody>
</table>

Phenylephrine pressor response (ΔAO Press)

Doses 1, 2, and 3 in dogs given yohimbine were 0.5, 1.0, and 2.0 mg/kg (cumulative); doses 1, 2, and 3 in ketanserin-treated dogs were 0.25, 0.50, and 1.0 mg/kg (cumulative). Three μg/kg phenylephrine (single iv bolus) was given before and 10 minutes after dose 2 was given. Press = pressure; Pre-Rx, Post-Rx = before and after yohimbine or ketanserin administration, respectively.

* Significantly lower than control value.
† Significantly lower than control value and values at other time points (P < .05 ANOVA, Duncan’s multiple range test).

Blood cell, and white blood cell aggregation. Damage to or disruption of the endothelial lining of the coronary vessel at and adjacent to the stenosis probably is an important stimulus for platelet aggregation in this model (Moncada and Vane, 1979; Bush et al., 1984). Traumatization of the vessel’s endothelium probably resulted from surgical manipulation of the artery, as well as placement of the cylindrical constrictor. We have observed, as have Fols, Aiken, and coworkers, that CBF declines to lower levels and at a faster rate in dogs in which the coronary arterial segment is damaged in some fashion (Aiken et al., 1979; Folts et al., 1982). These maneuvers most likely cause removal of some endothelium (a naturally antiaggregatory surface), exposing the underlying collagen, a strong attachment and proaggregatory stimulus for platelets (Moncada and Vane, 1979).

We evaluated a separate group of control animals that received saline (vehicle for all drugs tested except prazosin) to determine whether changes in the frequency of CFR occur spontaneously. The
frequency of CFR was nearly identical before and after saline, and the severity of the CFR, as judged by the mean of the three lowest values for CBF, also did not change significantly. Thus, as previously demonstrated (Uchida et al., 1975; Folts et al., 1976), within each dog, the CFR observed in this model are stable for at least 2 hours, allowing meaningful pre- vs. post-drug comparisons to be made.

Previous studies have shown that epinephrine stimulates platelet aggregation in vitro by stimulating α-adrenergic receptors, thereby lowering intraplatelet cAMP levels (Mitchell and Sharp, 1964; Mills, 1974). Also, epinephrine and norepinephrine (but not isoproterenol), in subaggregating concentrations, potentiate the aggregatory response to ADP, which also is released by platelets during activation and aggregation (Mills and Roberts, 1967). In radioligand-binding studies, α2-adrenergic receptors have been demonstrated in platelets (Hsu et al., 1979; Mukherjee et al., 1981). Platelets also accumulate, store, and release epinephrine during the release reaction accompanying aggregation (Mills, 1974). Results from several studies suggest that selective α2-adrenergic blockade inhibits platelet aggregation (Grant and Scutton, 1979). Results obtained from the present study seem to confirm this possibility in vivo. Levine (1973) demonstrated that cigarette smoke enhances the aggregatory response of human platelets to ADP in vitro, an effect which he ascribed to nicotine-induced increases in circulating catecholamine levels. In support of this, Folts and Bonebrake (1982) have shown that cigarette smoke inhaled by open-chest, anesthetized dogs with coronary constrictions increases the severity and frequency of CFR. They also showed that the exacerbation of CFR by cigarette smoke was accompanied by elevations in plasma catecholamine levels, that nicotine infusion itself can cause CFR, and that both are blocked by the nonselective α-adrenergic antagonist, phentolamine (Folts and Bonebrake, 1982). Finally, Pfister and Imhof (1977) have shown that orally administered phentolamine inhibits epinephrine-induced aggregation of human platelets in vitro.

In the present study, yohimbine, at a dose of 1 mg/kg, abolished ongoing CFR in approximately one-third of the dogs in which it was tested. A higher dose (2 mg/kg) was less effective and caused significant hypotension (Tables 2 and 3). Platelets contain epinephrine (Weil-Malherbe and Bone, 1958) and may represent an important local source of epinephrine for stimulation of platelet aggregation. This may provide a local decrease in epinephrine concentration that might not be reflected in plasma catecholamine measurements (Thomas, 1968; Mills, 1974). Although we cannot exclude the possibility that yohimbine was at least partially effective in reducing CFR by antagonizing serotonin responses (Kaumann et al., 1983; Robertson, 1983), we have shown that yohimbine administration in vivo does not prevent serotonin enhancement of ADP aggregation of platelets in vitro (Fig. 5). Therefore, we believe that ketanserin and yohimbine are acting, at least in part, through different mechanisms in abolishing and attenuating CFR, respectively, in this model.

The relatively selective α1-antagonist, prazosin, had no effect on CFR in this model, but inhibited the vasoprotective effects of phenylephrine. Similarly, propranolol did not alter CFR at doses of 1–2 mg/kg. It has been shown that propranolol exerts antithrombotic effects, which have been attributed to non-β-adrenergic blocking properties, since these effects are observed only at high doses and the inactive isomer, d-propranolol, is nearly equipotent to the l-isomer (Mills and Roberts, 1967; Bygdeman and Johnsen, 1969; Campbell et al., 1981).

Although it is known from in vitro studies that platelets accumulate, store (mostly in their dense granules), and release serotonin during aggregation (Baumgartner and Born, 1968; Mills, 1974; DeClerck et al., 1982), a major role in platelet aggregation in vivo has not been ascribed to serotonin. It was somewhat surprising to us, therefore, that ketanserin uniformly abolished CFR. Ketanserin’s ability to abolish CFR correlates with in vitro results reported recently by DeClerck et al (1982). Although serotonin is able to stimulate platelet aggregation directly by stimulation of platelet receptors (DeClerck et al., 1982; Janssen, 1983), it is believed to exert an equally important role in aggregation by “amplifying” the proaggregatory effects of other, more potent substances such as ADP and epinephrine. This effect is also mediated via 5HT1 receptor stimulation (DeClerck et al., 1982). In the present study, development of CFR in severely stenosed coronary arteries was associated with a significant elevation of TXB2 concentration. Ketanserin tended to decrease the distal coronary artery TXB2 values, although it did not return them to control values. In contrast, we have recently reported that administration of the selective thromboxane synthetase inhibitor, dazoxiben (UK-37,248-01), also reduces CFR and lowers distal coronary arterial TXB2 concentrations to control, preconstriction values (Bush et al., 1984). The data obtained in the present study suggest that the beneficial effect of ketanserin is not due solely to inhibition of platelet TXA2 production, and the inhibition of TXB2 production may be a consequence of the inhibition of platelet aggregation.

Ketanserin also possesses α1-adrenergic blocking effects (Brazenor and Angus, 1982), which probably contributed in part to its hypotensive effects. These hypotensive effects were observed in dogs with or without coronary constrictions (Tables 2 and 3). A vasodilatory effect probably did not contribute to ketanserin’s ability to abolish CFR, since the α1-antagonist, prazosin, which produced a comparable reduction in aortic pressure, was ineffective in this model. Furthermore, the lower dose (0.25 mg/kg) of
ketanserin, which caused only small declines in arterial pressure, was as effective as the higher dose in abolishing CFR.

In this model, ketanserin appeared to be more effective than yohimbine in abolishing CFR. Perhaps, ketanserin’s apparent superiority over yohimbine in abolishing CFR is related to its ability to inhibit the amplification by serotonin of epinephrine’s and ADP’s proaggregatory effects, as well as those of serotonin itself. Minsker et al. (1974) and Stone et al. (1961) have shown that the antihistaminic, antiserotonergic drug, cyproheptadine, inhibits the proaggregatory effects in vitro of ADP, thrombin, collagen, and serotonin.

In summary, the relatively selective a2-adrenergic and serotonin antagonists, yohimbine and ketanserin, reduce and essentially abolish, respectively, cyclic flow reductions frequently associated with platelet aggregation in stenosed canine coronary arteries. These results suggest that, in this model, catecholamines and serotonin may both influence platelet aggregation and coronary blood flow in severely narrowed coronary vessels.

We wish to acknowledge the expert technical assistance of Michael Deguchi, Janice McNatt, Cora Marsaw, Judy Ober, Gifford Ramsey, and Mary Beth Santowski, as well as the statistical assistance of Rebecca Lundswick. We also wish to thank Laurie Christian and Nancy Dickey for excellent secretarial assistance.

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West Point, Pennsylvania 19486. Dr. Campbell is the recipient of NIH K04 HI 00801.


INDEX TERMS: Coronary arterial stenosis • Thromboxane • Serotonin • Adrenergic antagonists • Cyclic coronary blood flow alterations
The effects of alpha 2-adrenergic and serotoninergic receptor antagonists on cyclic blood flow alterations in stenosed canine coronary arteries.

Circ Res. 1984;55:642-652
doi: 10.1161/01.RES.55.5.642

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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