High-dose Droperidol Protects against Experimental Coronary Thrombosis in Dogs and Pigs and Attenuates Aggregation of Porcine Platelets and Ca\(^{2+}\) Mobilization in Human Platelets

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**Background:** Activation of platelets after contact with thrombogenic substrates may be an early factor leading to coronary artery thrombosis and myocardial infarction. Halo- peridol, a butyrophenone, possesses weak in vitro platelet inhibitory activity.

Experiments were designed to determine whether droperidol, a butyrophenone adjunct to anesthesia, protected against experimental coronary thrombosis in intravenously anesthetized open-chest dogs and pigs, attenuated *ex vivo* porcine platelet aggregation, and inhibited agonist-induced increases in [Ca\(^{2+}\)] of human platelets.

**Methods:** In dogs and pigs, a lesion consisting of endothe- lialization, deep vessel wall injury, and critical stenosis was created in the proximal circumflex arteries, resulting in coronary thrombus formation accompanied by decreased circumflex artery blood flow. Embolization of the thrombus restored flow, but the cycle then repeated, resulting in repetitive cyclical flow reductions (CFRs). These were measured using an electromagnetic flow probe.

**Results:** In dogs, droperidol 0.2 mg/kg intravenously rapidly abolished CFRs in all ten animals, with frequency decreasing from 0.22 ± 0.01 cycles/min to 0. Droperidol 0.8 mg/kg intravenous rapidly abolished CFRs in seven of eight pigs, with frequency decreasing from 0.15 ± 0.01 to 0.02 cycles/min (*P* < 0.005). In both species, additional doses of droperidol were effective against CFRs augmented with intravenous epinephrine, a catecholamine that stimulates thrombosis. *Ex vivo* platelet aggregation studies were performed in platelet-rich plasma obtained from pigs before and after droperidol 0.8 mg/kg intravenous. Pretreatment with the drug resulted in marked inhibition of aggregation evoked by collagen, modest attenuation of that elicited by adenosine diphosphate (ADP), but no effect on that evoked by arachidonic acid. In human platelets, apparent [Ca\(^{2+}\)] was estimated using the fluorescent indicator indo-1 and flow cytometry. Droperidol 10\(^{-7}\), 10\(^{-6}\), and 10\(^{-5}\)M had a dose-dependent inhibitory effect on the amplitude of increases in [Ca\(^{2+}\)], evoked by 10\(^{-7}\)M serotonin (plus 10\(^{-7}\)M epinephrine). The higher droperidol concentration decreased the response to as much as 30% of control (*P* < 0.001). Droperidol lacked effect on Ca\(^{2+}\) mobilization elicited with 10\(^{-4}\)M ADP.

**Conclusions:** The results from three experimental models indicate that droperidol attenuates experimental coronary thrombosis in animals and suggest that this inhibition may result, in part, from a direct droperidol depressant effect on platelet activation and aggregation. (Key words: Anesthetics, intravenous: droperidol. Arteries, coronary: thrombosis. Blood, platelets: function; intracellular Ca\(^{2+}\). Hearts: coronary occlusion. Sympathetic nervous system, catecholamines: epinephrine.)

CORONARY thrombosis is the major cause of myocardial infarction in humans.\(^{1-4}\) A precipitating event—perhaps increased sympathetic activity, coronary artery vasoconstriction, or myocardial ischemia—in combination with acute structural changes within a coronary artery, such as plaque fissuring or rupture, are believed to initiate coronary thrombosis. Evidence obtained by coronary angiography and at autopsy indicates that coronary thrombosis is a common, penultimate event preceding myocardial infarction.\(^{5,6}\) Platelets are a major culprit.\(^{1-4}\) Their activation, on contact with thrombo- genic substrates within an atherosclerotic coronary artery wall, such as von Willebrand's factor and collagens, results in the self-sustaining, self-amplifying process.\(^{1-4}\) This thrombotic process is an important target for therapeutic intervention.\(^{4}\) However, despite considerable research effort to identify drugs with platelet...
stabilizing properties, surprisingly few are currently available for clinical use.

The current study was designed to test the hypothesis that droperidol, a butyrophenone tranquilizer and anesthetic adjunct, would inhibit experimental coronary thrombosis in animals, attenuate ex vivo platelet aggregation in porcine platelet-rich plasma, and perturb Ca^{2+} mobilization in human platelets. Droperidol was investigated because haloperidol, a closely related neuroleptic butyrophenone, possesses weak in vitro platelet inhibitory activity—a property also shared by the neuroleptic agents chlorpromazine and trifluoperazine.

Droperidol's effects on experimental coronary thrombosis were studied using a well-characterized open-chest canine model. This model, described originally by Folts et al. and subsequently adopted and modified by others, relies on a proximal circumflex coronary artery lesion consisting of critical stenosis, decidualization, and, in the current studies, deep vessel wall injury. This lesion triggers coronary thrombus formation that is accompanied by decreased coronary blood flow. At the nadir of the reduction in flow, the thrombus embolizes, restoring vessel patency and blood flow. However, the process then repeats, resulting in a second thrombus and its subsequent embolization. Repetitive cyclical flow reductions (CFRs) occur and may continue unabated for hours. Epinephrine, a catecholamine with a synergistic effect on platelet activity, may be infused intravenously to further augment CFRs. (In humans, catecholamines may be a link between stressful events and coronary thrombosis.)

To exclude a species-specific droperidol effect peculiar to dogs, experiments were also performed in pigs. Pigs were chosen because they have proven to be a useful model for investigating hemostatic and thrombotic disorders. In the current experiments, platelet aggregation studies were also performed using porcine platelet-rich plasma obtained before and after treatment of the animals with droperidol.

Platelet activation is a primary event in thrombosis and is, in part, initiated and sustained by elevation of platelet [Ca^{2+}]. Whether droperidol perturbed agonist-induced Ca^{2+} mobilization was addressed using platelets obtained from healthy human volunteers. The platelets were stimulated with serotonin (5-HT) and adenosine diphosphate (ADP), two agonists with a role in generating CFRs in dogs that may also participate in coronary thrombosis in humans. Increases in apparent [Ca^{2+}] were estimated in platelets loaded with the fluorescent Ca^{2+}-sensitive indicator indo-1 and flow cytometry.

**Materials and Methods**

All studies were approved by appropriate institutional review boards and performed in a humane manner.

**Surgical Preparation (Dogs and Pigs)**

Mongrel dogs (22–26 kg) were anesthetized with intravenous sodium pentobarbital, approximately 50 mg/kg, during the course of the experiment. Their lungs were mechanically ventilated (Harvard respirator, Millis, MA) with oxygen-air (P_{O_2}, 0.7). In each dog, a catheter was placed in a carotid artery for aortic pressure monitoring and in a jugular vein for drug and fluid administration. A left, fifth intercostal space thoracotomy was performed and the heart suspended in a pericardial cradle. A 2–3-cm proximal segment of circumflex coronary artery was exposed and nearby branches tied when necessary. A close-fitting electromagnetic flow probe (Spectra Med, Oxnard, CA) of appropriate size was placed around the artery and maintained perpendicular to the vessel. The "null" and "zero" controls were adjusted and periodically checked to ensure proper flowmeter operation. (Flow probes had been previously checked to ensure correct calibration.) An electrocardiographic lead was sutured to the epicardium in the region supplied by the circumflex artery. The model is outlined in figure 1. Body temperature was maintained using warming blankets. Arterial blood gases, arterial hemoglobin oxygen saturations, and urine output were monitored. Krebs-Ringer solution was infused intravenously.

Endothelial damage and deep vessel wall injury, with disruption of the lamina propria and rupture of the muscularis layer, were induced by squeezing the artery distal to the flow probe with cushioned forceps. A 4-mm-long plastic constrictor was placed around the damaged segment to achieve a critical stenosis. Fine adjustments were made by pulling a tapered fishline between the inside of the plastic constrictor and the outside of the vessel. (Critical stenosis, typically 60–70%, is that which permits resting blood flow to remain within the normal range but abolishes autoregulatory flow reserve. It results in absence of the reactive hyperemic response to a temporary 20-s occlusion of the vessel.) This experimental lesion produced spontaneous repetitive CFRs.
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Fig. 1. Creation of a critical stenosis in a segment of the circumflex coronary artery. A plastic cylinder, 4 mm in length, was placed around the coronary artery, en circleting and constricting the vessel and producing a 60–70% reduction in diameter. Tapered, nylon fishing line was placed between the inside wall of the plastic cylinder and the outside wall of the coronary artery. The line was pulled back and forth, permitting fine adjustments to the degree of stenosis. Coronary artery blood flow was measured with an electromagnetic flow probe placed around the vessel proximal to the stenosis. In addition, but not shown, the coronary artery endothelium was severely damaged and the vessel wall deeply injured.

Farm pigs (40–45 kg) were premedicated with 1 g intramuscular ketamine (Aveco, Fort Dodge, IA), anesthetized intravenously with ketamine 500 mg/h, fentanyl (USPC) 10 mg/h, and etomidate (Abbott) 10 mg/h—an anesthetic regimen resulting in stable hemodynamics. Their lungs were mechanically ventilated (Harvard respirator) with air-oxygen (Fio2 0.7). Monitoring was established as in dogs. However, access to the heart differed from that in dogs, requiring removal of a segment of chest wall that included skin, fat, muscle, and three ribs. Dissection of the circumflex coronary arteries from the underlying epicardium was performed with special care because pig coronary arteries are particularly fragile. Critical stenosis, deendothelialization, and deep vessel wall injury were established, again resulting in repetitive spontaneous CFRs.

Study Protocols

Dogs: Ten dogs were studied: 1) for 30 min to establish baseline frequency and characteristics of spontaneous CFRs; 2) for 30 min to determine whether bolus intravenous infusion of droperidol 0.2 mg/kg (LyphoMed, Rosemont, IL) abolished spontaneous CFRs; 3) for 15 min to determine whether challenge with epinephrine 0.4 µg·kg⁻¹·min⁻¹ intravenous restored CFRs after droperidol treatment; 4) for 30 min after the discontinuation of epinephrine and the addition of a second droperidol dose of 0.8 mg/kg administered over 10 min; and 5) for 15 min during rechallenge with epinephrine 0.4 µg·kg⁻¹·min⁻¹ to determine whether the additional dose of droperidol protected against CFRs augmented with epinephrine.

The droperidol doses chosen were those that effectively inhibited CFRs in pilot, albeit limited, studies. The epinephrine dose was that which has previously been shown to restore CFRs in dogs despite pretreatment with aspirin.14

Pigs: Eight pigs were studied: 1) for 30 min to establish baseline CFR frequency and characteristics of spontaneous CFRs; 2) for 15 min to establish baseline CFR frequency and characteristics during challenge with epinephrine 0.25 µg·kg⁻¹·min⁻¹ by intravenous infusion; 3) for 30 min (after discontinuation of the epinephrine infusion) to determine whether droperidol 0.8 mg/kg intravenous bolus infusion abolished spontaneous CFRs; and 4) for 15 min during further loading with intravenous infusion droperidol 0.2 mg/kg plus rechallenge with epinephrine 0.25 µg·kg⁻¹·min⁻¹ to determine whether the additional dose of droperidol protected against CFRs augmented with epinephrine.

The droperidol doses chosen were those that effectively attenuated CFRs in pilot studies. The epinephrine dose was that which augmented CFRs without causing severe ventricular ectopy.

Time-Control and Placebo Studies in Dogs: Five additional dogs were studied to demonstrate the continued presence and unchanged frequency of CFRs with time. Spontaneous CFRs were measured for 15 min and then for 15 min during epinephrine infusion (0.4 µg·kg⁻¹·min⁻¹). Epinephrine was discontinued and spontaneous CFRs monitored for a 2-h time-control period. To exclude investigator bias as a cause of droperidol effect, droperidol 0.2 mg/kg or placebo were then administered at the end of the time-control period, in a blinded randomized manner, using unmarked syringes. Cyclic flow reductions were observed for 30 min after each injection. (However, if the first syringe produced abolition of CFRs, the second syringe was not administered.)

Histology: Vessel wall injury was assessed in dog and pig coronary artery specimens obtained post mortem. Vessels were fixed in situ by perfusion with 2% glutaraldehyde and 1% paraformaldehyde in 0.1 molar cacodylate. Sections were stained with hematoxylin and eosin and with Heidenhain Weigert-Van Gieson's stain and examined using light microscopy. Some segments

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were coated with carbon and gold-palladium alloy and studied with scanning electron microscopy. Specimens were taken from damaged and undamaged regions of the vessels.

**Platelet Aggregation in Porcine Platelet-Rich Plasma**

Forty-five milliliters of blood were removed from each pig (n = 8) by fresh puncture of the left atrium with a 19-G needle immediately before and 10 min after intravenous infusion of droperidol 0.8 mg/kg. The first 5 ml were discarded to avoid contamination with tissue thromboplastin released during the needle puncture. The remaining blood was anticoagulated with 9:1 3.8% sodium citrate and platelet-rich plasma obtained by centrifugation (100 G, 15 min, 22°C). Platelets were stimulated with standard agonists: bovine collagen 15 and 30% solutions (Helena Laboratories, Beaumont, TX); ADP 5, 10, and 20 μM (Sigma, St. Louis, MO); and arachidonic acid 7.7 μM (Sigma).\(^{25,26}\) Light transmittance was measured in a dual-channel aggregometer (Peyton, Buffalo, NY) using the modified method of Born.\(^{22}\)

**Apparent Ca\(^{2+}\) in Human Platelets**

Forty-five milliliters of free-flowing venous blood was obtained from nine healthy, drug-free adult women and men. Special care was taken to exclude volunteers who recently took aspirin or other antiinflammatory agents. Platelet-rich plasma was obtained (above) and the platelet fraction spun, washed in Tyrode’s solution, and spun, and the pellet resuspended in buffer containing 0.1% azide and 2% fetal bovine serum. Platelets were counted (Coulter, Hialeah, FL) to obtain \(3 \times 10^8\) cells/ml. They were loaded with indo-1 10 μM (Molecular Probes, Eugene, OR) for 30 min at 37°C in the acetyoxymethyl ester (AM) form, which is hydrolyzed in the cytoplasm to the acidic cell impermanent form. The platelets were suspended in Tyrode’s solution, \(~5 \times 10^6\) cells/ml, and preincubated for 10 min with or without \(10^{-7}, 10^{-6}, \) or \(10^{-5}\) M droperidol (Sigma).

Changes in fluorescence ratio (representing changes in \([Ca^{2+}])\) were estimated using a flow cytometer (Beckton Dickinson FACStar Plus\(^{\circ}\)) in which platelets travel in single file in a fine stream at about 1000/s through a laser beam, resulting in excitation of indo-1 fluorescence. Excitation light (351–364 nm) is provided by an argon ion laser and dual emitted wavelengths are split and measured separately but instan-

taneously by two photomultiplier detectors. The shorter wavelengths, which originate from Ca\(^{2+}\)-bound indo-1, are measured as linear integrated signals after passing a 390 ± 5-nm bandpass filter. The longer wavelengths, which originate from Ca\(^{2+}\)-free indo-1, are measured using a 500 ± 20-nm bandpass filter. The instrument generates the ratio of the two fluorescence signals (395/500). Clumps of cells, aggregated platelets, and debris were identified by their forward low-angle light scatter and sidescatter light scatter profiles and rejected from analysis.

Platelet suspension (1.0 ml) was placed in the flow cytometer reservoir, baseline fluorescence ratios were established, and \(10^{-6}\) M ADP or \(10^{-7}\) M 5-HT (with or without \(10^{-7}\) M epinephrine) was added without interrupting the continuous flow of cells through the cytomter. Each individual experiment was accompanied by a control performed immediately afterward and in an identical manner but in the absence of droperidol. Fluorescence ratios were converted to apparent \([Ca^{2+}]\) values by exposing platelets acutely permeabilized with digitonin to different concentrations of extracellular Ca\(^{2+}\) and then using an established indo-1 \(K_D\) (250 nM at 37°C) and measuring and calculating the ratio of fluorescence of Ca\(^{2+}\)-free indicator and Ca\(^{2+}\)-bound indicator, maximum fluorescence ratio, and minimum fluorescence, solving the equations describing the relationships.\(^{28}\)

**Data Analysis:** In animal experiments, a single CFR was considered to be a decrease in coronary blood flow followed by restoration of flow. Changes in CFR frequency were measured. Each animal served as its own control. Porcine platelet aggregation is reported in arbitrary light transmittance units. Apparent \([Ca^{2+}]\) values obtained in human platelets are approximations, not absolute values. N refers to the number of individual animals except during flow cytometry, when n refers to the number of individual pairs of experiments, each consisting of a droperidol study immediately accompanied by its own control. (Platelets were obtained from nine donors.) Data were analyzed by Student’s paired \(t\) testing between droperidol and control groups. All values are reported as mean ± SEM.

**Results**

**Cyclical Flow Reductions**

**Dogs:** 1) All ten dogs demonstrated spontaneous, repetitive CFRs throughout an initial 30-min control pe-
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Table 1. Cyclical Flow Reductions, Coronary Blood Flow, and Hemodynamics in Dogs

<table>
<thead>
<tr>
<th></th>
<th>Dogs with CFRe</th>
<th>Dogs without CFRe</th>
<th>Hemodynamics (all dogs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>n</td>
<td>CFR Frequency (cycles/min)</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>10</td>
<td>0.22 ± 0.01</td>
</tr>
<tr>
<td>0.2 mg/kg droperidol</td>
<td>10</td>
<td>0</td>
<td>0°F</td>
</tr>
<tr>
<td>Challenge with epinephrine</td>
<td>10</td>
<td>9</td>
<td>0.25 ± 0.04</td>
</tr>
<tr>
<td>0.8 mg/kg droperidol</td>
<td>10</td>
<td>1</td>
<td>0.02 ± 0.02°F</td>
</tr>
<tr>
<td>Rechallenge with epinephrine</td>
<td>10</td>
<td>3</td>
<td>0.06 ± 0.03°F</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. CFR = cyclical flow reduction; epinephrine = Intravenous epinephrine 0.4 μg·kg⁻¹·min⁻¹.

* P < 0.05 vs. control.
† P < 0.01 vs. initial challenge with epinephrine.

period. 2) Droperidol 0.2 mg/kg intravenous abolished CFRs in all ten dogs. Onset of the droperidol effect was rapid, occurring within 2.2 ± 0.3 min of drug administration. Cyclical flow reductions did not reoccur during the ensuing 30-min study period. 3) Challenge with epinephrine 0.4 μg·kg⁻¹·min⁻¹ intravenous resulted in reoccurrence of CFRs in nine of the ten dogs. 4) Epinephrine was discontinued and dogs administered an additional droperidol 0.8 mg/kg intravenous (abolishing CFRs in all but one dog). 5) The dogs were rechallenged with epinephrine 0.4 μg·kg⁻¹·min⁻¹. Droperidol protected against recurrence of CFRs in all but three dogs, despite epinephrine infusion. Results are presented in table 1 and figure 2. The effects of droperidol on CFRs in an individual dog are shown in figure 3.

Pigs: 1) All eight pigs demonstrated spontaneous, repetitive CFRs during an initial 30-min control period. 2) Challenge with epinephrine 0.25 μg·kg⁻¹·min⁻¹ intravenous increased CFR frequency and magnitude (P = 0.05). Epinephrine was discontinued, and the preparation allowed to return to baseline generation of spontaneous CFRs. 3) Droperidol 0.8 mg/kg intravenous abolished the spontaneous CFRs in seven of the eight pigs. Onset of the droperidol effect was rapid in the seven droperidol sensitive animals, occurring within 0.5 ± 0.3 min of drug administration and persisting throughout the 30-min study period. 4) Additional droperidol, 0.2 mg/kg, was administered by 15-

Fig. 2. Effects of droperidol on the frequency of cyclical flow reductions (CFRs) in intravenously anesthetized open chest dogs. Cyclical flow reductions occurred in all dogs during the control period. Droperidol 0.2 mg/kg abolished CFRs in all dogs. Challenge with intravenous epinephrine 0.4 μg·kg⁻¹·min⁻¹ restored CFRs. Additional droperidol 0.8 mg/kg decreased CFR frequency during rechallenge with epinephrine. (*P < 0.01 when control and droperidol treatment were compared and when epinephrine and epinephrine plus additional droperidol treatment were compared.) Data are mean ± SEM.

Fig. 3. Cyclical flow reductions and aortic blood pressure in an individual dog. Cyclical flow reductions (top left) were abolished (at right) after droperidol 0.2 mg/kg. BP = blood pressure; BF = blood flow.

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Table 2. Cyclical Flow Reductions, Coronary Blood Flow, and Hemodynamics in Pigs

<table>
<thead>
<tr>
<th></th>
<th>Pigs with CFRs</th>
<th></th>
<th>Pigs without CFRs</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>n</td>
<td>CFR Frequency (cycles/min)</td>
<td>Initial Coronary Blood Flow (ml/min)</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>8</td>
<td>0.15 ± 0.01</td>
<td>52 ± 6</td>
</tr>
<tr>
<td>Challenge with epinephrine</td>
<td>8</td>
<td>8</td>
<td>0.18 ± 0.02</td>
<td>52 ± 4</td>
</tr>
<tr>
<td>0.8 mg/kg droperidol (epinephrine off)</td>
<td>8</td>
<td>1</td>
<td>0.02 ± 0.02*</td>
<td>40</td>
</tr>
<tr>
<td>0.2 mg/kg plus droperidol rechallenge with epinephrine</td>
<td>8</td>
<td>2</td>
<td>0.05 ± 0.03†</td>
<td>41 ± 3</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. CFR = cyclical flow reduction; epinephrine = intravenous epinephrine 0.25 μg·kg⁻¹·min⁻¹.

* P < 0.005 vs. control.
† P < 0.005 vs. initial challenge with epinephrine.

min intravenous infusion and the pigs rechallenged with epinephrine 0.25 μg·kg⁻¹·min⁻¹. Droperidol protected against CFRs in all but two animals, despite epinephrine infusion. Data are shown in table 2 and figure 4.

**Time Control and Placebo Studies in Dogs:** All five dogs demonstrated spontaneous CFRs that increased in frequency when challenged with epinephrine 0.4 μg·kg⁻¹·min⁻¹ intravenously. Epinephrine was discontinued with return to baseline CFR frequency. Spontaneous CFRs continued unabated in all five dogs during the following 2 h. Randomized blind administration of placebo had no effect on CFR frequency but droperidol 0.2 mg/kg intravenous abolished CFRs in all animals. Data are shown in table 3.

**Coronary Artery Histology:** Damaged segments of dog and pig coronary arteries demonstrated loss of endothelium, disruption of the internal elastic lamina, and rupture of the muscularis mucosa. Figure 5 demonstrates the appearance of a normal and a damaged coronary artery segment from the same pig. Scanning electron microscopy demonstrated loss of endothelial cells, disrupted subendothelial tissue, and ruptured connective tissue fibrils.

**Platelet Aggregation in Porcine Platelet-Rich Plasma**

Aggregation responses to collagen, ADP, and arachidonic acid were measured in platelet-rich plasma obtained from pigs before and 10 min after droperidol 0.8 mg/kg. All samples aggregated in response to the agonists. Aggregation evoked by collagen was markedly inhibited in samples containing droperidol, while that evoked by ADP was modestly inhibited. In contrast, responses to arachidonic acid were unchanged by droperidol pretreatment. Data are shown in table 4.

**Apparent [Ca²⁺]** in Human Platelets

Baseline [Ca²⁺] in quiescent, unstimulated platelets did not differ between untreated control platelets and

![Fig. 4. Effects of droperidol on the frequency of cyclical flow reductions (CFRs) in intravenously anesthetized open chest pigs. Cyclical flow reductions occurred spontaneously in all animals. Frequency of CFRs increased on challenge with intravenous epinephrine at 0.25 μg·kg⁻¹·min⁻¹ (P = 0.05). Epinephrine was discontinued with persistence of spontaneous CFRs. Droperidol 0.8 mg/kg decreased their frequency (†P < 0.005 in comparison with control). Additional droperidol 0.2 μg/kg decreased CFR frequency during rechallenge with epinephrine (‡P < 0.005 in comparison with CFRs during epinephrine infusion).](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931320/)
Table 3. Cyclical Flow Reductions, Coronary Blood Flow, and Hemodynamics in Dogs during Time-control and Droperidol-placebo Studies

<table>
<thead>
<tr>
<th></th>
<th>Dogs with CFRs</th>
<th>Initial Coronary Blood Flow (mL/min)</th>
<th>Coronary Blood Flow at Nadir of CFR (mL/min)</th>
<th>Dogs without CFRs</th>
<th>Hemodynamics (all dogs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>n</td>
<td>CFR Frequency (cycles/min)</td>
<td></td>
<td>n</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>0.23 ± 0.04</td>
<td>60 ± 5</td>
<td>7 ± 1</td>
<td>50 ± 8</td>
</tr>
<tr>
<td>Challenged with epinephrine</td>
<td>5</td>
<td>0.35 ± 0.05*</td>
<td>50 ± 8</td>
<td>9 ± 6</td>
<td>95 ± 4</td>
</tr>
<tr>
<td>Time-control period (2 h)</td>
<td>5</td>
<td>0.25 ± 0.02</td>
<td>65 ± 3</td>
<td>10 ± 3</td>
<td>102 ± 4</td>
</tr>
<tr>
<td>Placebo</td>
<td>3‡</td>
<td>0.23 ± 0.03</td>
<td>63 ± 9</td>
<td>6 ± 4</td>
<td>94 ± 8</td>
</tr>
<tr>
<td>0.2 mg/kg droperidol</td>
<td>5</td>
<td>0</td>
<td>—</td>
<td>5</td>
<td>27 ± 4</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. CFR = cyclical flow reduction; ND = not determined; epinephrine = intravenous epinephrine 0.4 µg · kg⁻¹ · min⁻¹.
* P < 0.05 vs. control.
† P < 0.05 vs. time-control.
‡ Three dogs received placebo.

Platelets preincubated for 10 min with 10⁻⁷, 10⁻⁶, or 10⁻⁵M droperidol.

Platelets were stimulated with 10⁻⁵ M 5-HT, an endogenous Ca²⁺ mobilizing agonist.²⁵,²⁶,³³ Platelets from some, but not all, donors responded with a rapid and marked increase in [Ca²⁺], followed by a more gradual return to baseline. Preincubation with 10⁻⁵ M droperidol decreased the amplitude of the Ca²⁺, response from 122 ± 19 nM (controls) to 30 ± 2 nM (P < 0.05; n = 4). (Difficulties in identifying donors with 5-HT responsive platelets limited data collection to four experiments.)

To obtain consistent responsiveness to 5-HT without screening large numbers of donors for 5-HT sensitive platelets, all subsequent experiments were performed only in the presence of 10⁻⁷ M epinephrine.²⁵,³⁴ Epinephrine increases platelet responsiveness to 5-HT but does not, by itself, elevate [Ca²⁺].²⁵,³⁴ In the presence of 10⁻⁷ M epinephrine, 10⁻⁴ M 5-HT evoked an increase in [Ca²⁺], followed by a more gradual return to baseline in platelets from all donors.³⁴ Droperidol had a dose-dependent inhibitory effect on Ca²⁺ mobilization. The increase in [Ca²⁺], and the areas under the time-response curves were both decreased in samples preincubated with 10⁻⁵ M and with 10⁻⁴ M droperidol. Apparent [Ca²⁺], values are presented in table 5, traces from typical individual experiments are shown in figure 6, and percent of inhibition of the response indicated using integrated areas under the time-response curves are shown in figure 7.

In contrast to the effects of droperidol on responses evoked by 5-HT, droperidol had no statistically significant effect on [Ca²⁺], responses evoked by 10⁻⁵ M ADP, also an endogenous Ca²⁺ mobilizing agonist.²⁵,²⁶ Data are shown in table 5 and figure 7.

Discussion

The results indicate that droperidol attenuates CFRs, an experimental indicator of coronary thrombosis, in dogs and pigs. Results also suggest that this inhibition may result, in part, from a direct droperidol depressant effect on platelet activation and aggregation.

Studies were pursued in vivo using an established open-chest animal model. For almost 20 yr, this well-characterized and versatile animal model has been used both with and without modifications to demonstrate interventions that accelerate or inhibit experimental coronary thrombosis. The effectiveness of a wide range of antiplatelet drugs has been demonstrated, including that of aspirin,¹⁰ prostacyclin,¹² combined treatment with thromboxane and 5-HT receptor antagonists,¹⁶ and monoclonal antibodies directed against platelet GPIIb-IIIa adhesive receptors.¹³ Epinephrine, in contrast, is prothrombotic.¹⁴ Cyclical flow reductions cannot be halted by coronary artery vasodilators.¹¹ The model generates repetitive CFRs, which are regarded as a satisfactory indicator of active coronary thrombosis. The preparation is stable with time, with CFRs persisting for up to 3 h in open-chest dogs and for 3 days or longer in chronically instrumented awake dogs.³,¹⁸,¹⁹ This stability, which is important when animals serve as their own controls, was reconfirmed in the current study when, in the absence of interventions, CFRs were...
sustained without interruption for 2 h. In addition, lack of responsiveness to placebo, but sensitivity to droperidol, was established in a randomized, blinded manner.

In the current experiments, the generation of CFRs was dependent on a complex, mechanical lesion created within the proximal circumflex coronary arteries. This lesion exhibited three key prothrombotic elements: endothelial damage, deep vessel wall injury, and critical stenosis. Endothelial damage results in loss of endothelial-derived platelet stabilizing factors, such as nitric oxide and prostacyclin, and also exposes blood to thrombogenic subendothelial smooth muscle and collagen. Deep vessel wall injury, with disruption of the lamina propria and rupture of the muscularis mucosa, provides an extensively damaged prothrombotic surface that permits firm attachment of the developing thrombus. Damage also mimics deep fissuring commonly present in human coronary artery lesions. (However, in dogs and pigs, atherosclerotic changes were not present.) Critical stenosis causes turbulence and increases wall shear rate. This is a measure of the difference in velocity of flow between the center and periphery of the blood flow profile, which distributes platelets toward the periphery—factors which predispose to platelet aggregation. Thrombus resulting from the experimental lesion is composed primarily of aggregated platelets, but also includes red blood cells and leukocytes.

Droperidol rapidly and effectively attenuated CFRs in both dogs and pigs. The degree of protection against CFRs was particularly profound considering that droperidol is primarily an anesthetic adjunct rather than a specific antiplatelet agent. However, the doses required were relatively high—0.2 mg/kg for dogs and 0.8 mg/kg in pigs. Surprisingly, in both species, these doses were not associated with hemodynamic instability. At higher dosage, droperidol also attenuated CFRs augmented by epinephrine. Epinephrine has prothrombotic effects in vivo, acting in part by increasing platelet responsiveness to other platelet-active agents. Epinephrine also causes resistance to platelet stabilizing therapies, decreasing the effectiveness of platelet inhibitors, including aspirin, enzymes that break down ADP, and thromboxane receptor antagonists. Consequently, protection against CFRs during infusion of epinephrine was a relatively stringent test of droperidol’s effectiveness.

Platelet aggregation studies were performed in platelet-rich plasma obtained from pigs before and after droperidol 0.8 mg/kg. In this model, aggregation is dependent on both platelet function and hemostatic factors present in the plasma. Droperidol inhibited aggregation evoked by collagen, a noteworthy inhibitory effect, because vessel wall collagens have an important role in experimental thrombosis and are presumed to participate in triggering thrombosis in humans. Droperidol modestly inhibited aggregation induced by ADP and had no effect on that elicited by arachidonic acid.

Fig. 5. A: Elastin (Van Giesen) stained cross-section specimen removed from the undamaged segment of pig left anterior descending coronary artery. Intact internal elastic lamina and normal coronary architecture are apparent. Magnification: 40X. B: Elastin (Van Giesen)-stained cross-section specimen removed from a stenosed and damaged segment of the circumflex coronary artery from the same pig. The darkly stained internal elastic laminar is ruptured. Damage extends into the muscularis mucosa. Adherent thrombus can be seen in the vessel lumen. Magnification: 40X.
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Table 4. *Ex Vivo* Platelet Aggregation in Platelet-rich Plasma Obtained from Pigs before and after 0.8 mg/kg Droperidol

<table>
<thead>
<tr>
<th></th>
<th>Platelet Aggregation (Light Transmittance Units)</th>
<th>Adenosine Diphosphate</th>
<th>Arachidonic Acid (7.7 µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Collagen 15% 30%</td>
<td>5 µM</td>
<td>10 µM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predroperidol</td>
<td>14 ± 3</td>
<td>19 ± 2</td>
<td>25 ± 3</td>
</tr>
<tr>
<td>(control)</td>
<td>33 ± 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postdroperidol</td>
<td>7 ± 2*</td>
<td>15 ± 4</td>
<td>20 ± 4†</td>
</tr>
<tr>
<td></td>
<td>20 ± 6†</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n = 6 pigs.

* P < 0.05 vs. control.
† P < 0.01 vs. control.

Absence of effect on aggregation induced by arachidonic acid, an agonist that acts in part by serving as a substrate for thromboxane synthesis *via* cyclooxygenase, indicates that the mechanism of droperidol action is not cyclooxygenase inhibition.

Platelet Ca\(^{2+}\) mobilization is a key event in initiating platelet activities such as shape change, granule secretion, and adhesive receptor expression. Ca\(^{2+}\) mobilization was studied in human platelets using flow cytometry. Platelets were stimulated with either 5-HT or ADP, two agonists with roles in platelet activation in the human circulation. In addition, the two agonists are, in part, responsible for CFRs in dogs. In human platelets, however, 5-HT does not consistently evoke marked increases in [Ca\(^{2+}\)] in the platelets of all donors. In contrast, in the presence of epinephrine, platelet responses to 5-HT are increased. (Epinephrine itself is not a Ca\(^{2+}\) mobilizing agonist.) This synergism may involve interactions at the receptor-G-protein level of signal transduction. Consequently, only limited data were collected when platelets were stimulated solely with 5-HT, but demonstrated that droperidol 10\(^{-5}\)M potently inhibited the increase in Ca\(^{2+}\). Droperidol dose-response relationships were probed in the presence of 10\(^{-7}\)M epinephrine, conditions perhaps better representative of those *in vivo*, and demonstrated a dose-dependent inhibitory effect. Interestingly, dose-dependent depression of responses evoked by 5-HT have also been demonstrated *in vivo* in guinea pigs, when droperidol attenuated bronchoconstriction evoked by 5-HT. In the current experiments, it must be noted that, although 10\(^{-5}\)M droperidol substantially decreased Ca\(^{2+}\) responses, this *ex vivo* droperidol concentration would be grossly supratherapeutic *in vivo*.

Droperidol did not depress Ca\(^{2+}\) responses in human platelets elicited by 10\(^{-6}\)M ADP, an observation somewhat surprising, because droperidol modestly inhibited

Table 5. The Effects of Preincubation with Droperidol on [Ca\(^{2+}\)], Responses in Human Platelets Evoked by 10\(^{-5}\) M 5-HT (+ 10\(^{-7}\) M Epinephrine) or by 10\(^{-6}\) M Adenosine Diphosphate (ADP)

<table>
<thead>
<tr>
<th></th>
<th>Ca(^{2+}) Response Evoked by 10(^{-5}) M 5-HT (+ 10(^{-7}) M Epinephrine)</th>
<th>Ca(^{2+}) Response Evoked by 10(^{-6}) M ADP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline [Ca(^{2+})] (nm)</td>
<td>Peak [Ca(^{2+})] (nm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>11</td>
<td>89 ± 4</td>
</tr>
<tr>
<td>10(^{-7}) M droperidol</td>
<td>92 ± 4</td>
<td>163 ± 16</td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>85 ± 4</td>
</tr>
<tr>
<td>10(^{-6}) M droperidol</td>
<td>92 ± 4</td>
<td>158 ± 26†</td>
</tr>
<tr>
<td>Control</td>
<td>11</td>
<td>86 ± 5</td>
</tr>
<tr>
<td>10(^{-5}) M droperidol</td>
<td>90 ± 6</td>
<td>128 ± 14†</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. N = number of individual experiments each consisting of control + droperidol treatment.

* P < 0.02 vs. control.
† P < 0.001 vs. control.

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ADP-induced aggregation in pig platelet-rich plasma. The results are not entirely discordant, because aggregation studies provide information concerning the sum effect of interacting prothrombotic pathways, while flow cytometry experiments are specific solely for [Ca^{2+}]. These data demonstrate that not all agonist-induced platelet Ca^{2+} responses are necessarily sensitive to droperidol.

Ca^{2+} mobilization in human platelets and CFRs in dogs and pigs remained sensitive to inhibition by droperidol, despite the presence of epinephrine. These data may suggest that α-adrenergic mechanisms are also a target for droperidol action. The platelets of most species possess few α₁-adrenergic receptors, the majority being of the α₂ type, which are linked to inhibition of cAMP formation. Their agonism promotes platelet activation. Droperidol has been shown to exhibit α₁-adrenergic receptor inhibition in canine blood vessels. If it were to possess α₂-antagonist activity as well, platelet stability would be enhanced. However, such an explanation would not completely explain droperidol's antithrombotic action in vivo, because α-adrenergic blockade does not have marked effects on CFRs in this experimental model.

The current in vivo experiments were performed in intravenously anesthetized animals. In dogs, background anesthesia with pentobarbital may have confounded the results because, in some species, barbiturates have a platelet stabilizing effect. However, droperidol also inhibited CFRs in pigs anesthetized with fentanyl, ketamine, and etomidate—drugs not known to possess antiplatelet activity.

In summary, results demonstrate that droperidol exerts marked protection against CFRs in dogs and pigs.

![Graph](http://anesthesiology.pubs.asahq.org)

**Fig. 7.** Agonist-induced increases in [Ca^{2+}] after preincubation with 10^{-5}, 10^{-6}, or 10^{-3} M droperidol (10 min). Data are expressed as percent of control (100%) using integrated areas under the time-response curves. Responses were evoked by 10^{-7} M serotonin (5-HT, left) in the presence of 10^{-7} M epinephrine or by 10^{-7} M adenosine diphosphate (ADP, right). (*P* < 0.02 when compared with control, ††P < 0.001 when compared with control.)

Droperidol attenuated porcine platelet aggregation evoked by collagen and, to a lesser degree, that elicited by ADP. In human platelets, droperidol had a dose-dependent depressant action on Ca^{2+} mobilization evoked by 5-HT (in the presence of epinephrine). Although effects were observed at high droperidol doses, in animals, the actions of droperidol were rapid and consistent, and resulted in considerable amelioration of experimental coronary thrombosis. The etiology of droperidol's actions remains to be determined.

### References


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