Primary visceral afferent fibers are present in both vagus and sympathetic nerves. Activation of these afferents produces differential reflex effects. We and others (5, 9, 32) have shown that excitation of cardiac and pulmonary vagal afferents electrically or chemically evokes reflex decreases in mean arterial pressure (MAP) and heart rate (HR). Conversely, excitation of cardiac sympathetic afferent fibers evokes reflex increases in blood pressure (20, 24, 26, 32). Results of previous studies from our laboratory have indicated that excitation of abdominal sympathetic afferents electrically or chemically also produces reflex increases in blood pressure and HR (25, 28, 30).

Phenylbiguanide (PBG) has been used as a "selective" agonist to identify cardiac and pulmonary vagal C fibers and the associated reflex bradycardia and depressor responses (i.e., Bezold-Jarisch reflex) for over 40 years. The location of receptors mediating the inhibitory cardiovascular and respiratory responses to PBG infusion has been the focus of intense study. In general, it has been suggested that respiratory responses to PBG infusion are mediated by receptors on vagal nerve endings in the lungs, whereas the hypotension and bradycardia are mediated by receptors on vagal nerves innervating both the lungs and heart (5). There have been no studies that have focused on the possibility that PBG might act on sympathetic afferents to produce excitatory cardiovascular responses. In this regard, although Evans and co-workers (9) reported that injection of PBG into the proximal aorta produces tachycardia and hypertension, they did not identify the substrate underlying this response. Recently, we (12, 13) found that 5-HT (serotonin), produced during abdominal ischemia, stimulates sympathetic afferents. Because PBG mimics the selective action of 5-HT on mammalian neurons (8, 10, 16), it is possible that this agonist stimulates sympathetic afferents leading to the tachycardia and pressor responses.

5-HT3 receptors are present on both vagal and sympathetic afferent nerve fibers (9, 12, 17). PBG is a 5-HT3 receptor agonist capable of stimulating cardiac and pulmonary chemosensitive receptors with vagal afferents to elicit the Bezold-Jarisch reflex (31). 5-HT3 receptors also are located on ischemically sensitive sympathetic afferent nerve endings (12). Blair et al. (4) observed that the excitatory effects of PBG on spinal neurons are mediated by 5-HT3 receptors on cardiac sympathetic afferents. In aggregate, these data indicate that PBG has the potential to stimulate sensory nerves in different regions of the body. There is, however, no information regarding the action of PBG on sympathetic afferent endings outside the thoracic area. The potential exists for PBG stimulating both visceral vagal and sympathetic afferents, through a 5-HT3 receptor mechanism, to produce differential hemodynamic effects according to the site of its administration. Therefore, the goals of this study were to test the hypotheses that: 1) hemodynamic response to PBG varies according to the location of its administration; 2) arterial administration of PBG evokes a reflex excitatory cardiovascular response, in part by activating ischemically sensitive abdominal sympathetic afferents; and 3) activation of abdominal sympathetic afferents by PBG through a 5-HT3 receptor mechanism leads to the reflex pressor response.

METHODS

Surgical Preparation

Experiments were performed on fasted adult cats of either sex (3.2 ± 0.1 kg). Surgical and experimental protocols used
in this study were approved by the Animal Use and Care Committee at the University of California at Davis. The studies conformed to APS Guidelines and Principles Involving Animals. Anesthesia was induced with ketamine (20–30 mg/kg im) and maintained with α-chloralose (40–50 mg/kg iv). Additional injections of α-chloralose (5–10 mg/kg iv) were given as needed to maintain an adequate depth of anesthesia. The trachea was intubated and respiration maintained artificially (model 661, Harvard ventilator, Ealing, South Natick, MA). The cat was ventilated with 100% O2 through the respirator. A femoral vein was cannulated for administration of drugs and fluid. A femoral arterial catheter was positioned with the tip positioned in the descending thoracic aorta (above the diaphragm) for measurement of pressure and regional intra-arterial administration of drugs. For one protocol, another catheter (PE-90, Clay Adams, Parsippany, NJ) was inserted into the left ventricle (LV) through the left carotid artery for administration of drugs. Systemic arterial blood pressure and HR were measured by a pressure transducer (Statham P231D, Gould, Cleveland, OH) connected to the femoral arterial catheter. We frequently assessed arterial blood gases with a blood gas analyzer (model ABL-3, Radiometer) and maintained them within physiological limits (PO2 > 100 mmHg, Pco2 28–35 mmHg, pH 7.35–7.45) by adjusting 100% O2 as needed to maintain an adequate depth of anesthesia. 

Afferent Recording

The surgical preparation used for recording single-unit activity of abdominal sympathetic C fiber afferents has been described previously (14). In brief, a midline sternotomy was performed. The third through eleventh right ribs and the middle and caudal lobes of the right lung were removed. Both phrenic nerves were isolated and cut. The fascia overlying the middle and caudal lobes of the right lung were removed. Both described previously (14). In brief, a midline sternotomy was performed. A water heating pad and a heat lamp. Rectal thermistor and maintained at 36–38°C with a circulator, Beaverton, OR), and then recorded on a chart recorder (Audiomonitor, Grass) and processed through an audioamplifier (AM8B, Tektro, OR) and oscilloscope (model 2201, Tektronix, Beaverton, OR), and then recorded on a chart recorder (model TA 4000B, Gould). The neurogram also was fed into an IBM-compatible Pentium computer through an analog-to-digital interface card (R. C. Electronics, Santa Barbara, CA) for subsequent off-line analysis. The discharge frequency of afferents was analyzed using data acquisition and analysis software (EGAA, version 3.02, R. C. Electronics).

An inflatable occlusion cuff was placed around the descending thoracic aorta just above the diaphragm. A ventral midline incision was used to expose abdominal visceral organs. We closed the abdominal incision with towel clamps and covered the viscera with warm saline-soaked gauze to prevent fluid and heat losses. Receptive fields ofafferents were located precisely using a fine-tipped glass rod and a stimulating electrode to evoke the action potential of the afferent. We determined conduction time by measuring the interval from stimulation to the action potential of the afferent on the recording electrode. Conduction distance was estimated with a thread placed from the receptive field along the supposed afferent pathway through the prevertebral ganglion along the course of the major splanchnic nerve to the sympathetic chain and the recording electrode. C fibers and Aδ fibers were classified as those with a conduction velocity (CV) < 2.5 m/s and 2.5–30 m/s, respectively. Each afferent had a receptive field that could be located precisely. Afferents were considered to be ischemically sensitive if their discharge activity during 10 min of abdominal ischemia was increased at least twofold above baseline activity (14).

Experimental Protocols

Response of MAP and HR to PBG. In 10 closed-chest cats, PBG (40 µg/kg) was injected randomly into the LV, thoracic descending aorta, or femoral vein. This dose of PBG effectively stimulates cardiac chemosensitive receptors with vagal afferent pathways (21). We recorded MAP and HR responses during application of PBG. PBG (RBI, Natick, MA) was dissolved in 0.9% NaCl to a concentration of 1 mg/ml and was prepared fresh daily. Injection was done over a 2- to 3-s period. More than one injection usually was given to each cat. Each injection was separated by an interval of at least 30 min. No tachyphylaxis was observed during this protocol. The same volume of normal saline was injected into the LV intra-arterially and intravenously as a control.

Effects of denervation on response of MAP and HR to LV PBG. After identification of depressor responses following LV injection of PBG in closed-chest cats, we randomly divided the animals into one of two groups. In the first group (n = 4 cats), a bolus of PBG (40 µg/kg) was injected into the LV before and after bilateral cervical vagotomy, followed by bilateral cervical vagotomy plus celiac and superior mesenteric ganglionectomy, while we recorded HR and MAP. In the second group (n = 4), the same dose of PBG was injected into the LV before and after celiac and superior mesenteric ganglionectomy, followed by bilateral cervical vagotomy. The same volume of normal saline was injected into the LV as a vehicle control. Bradykinin (10 µg/ml) was applied to the gallbladder before celiac and superior mesenteric ganglionectomy to document that the animal remained reflexogenic (30). Conversely, absence of a pressor response to the application of bradykinin was used as the criterion for successful ganglionectomy.

To examine the reproducibility of blood pressure and HR responses to three repeated injections of PBG into the LV, a group of cats (n = 5) was treated identically as noted above but was not subjected to denervation.

Dose response. In five closed-chest animals, four applications of various concentrations of PBG, ranging from 10 to 100 µg/kg, were injected either intra-arterially or intravenously in a randomized fashion. The response of MAP to the application of PBG was recorded. Dose-response curves were generated with different doses of PBG applied at least 30 min apart to avoid tachyphylaxis.

Reproducibility. In seven closed-chest animals, PBG (40 µg/kg) was injected twice into the descending thoracic aorta over an interval of 30 min while the MAP response was recorded. Normal saline (2–3 ml) was injected intravenously between injections of PBG as the vehicle control for tropisetron.

Effects of 5-HT3 receptor blockade on MAP response to PBG. In six closed-chest cats, we examined the effect of blockade of 5-HT3 receptors with tropisetron on the MAP response to intra-arterial PBG. Tropisetron (RBI) was dissolved in 0.9% NaCl to a concentration of 2 mg/ml and was prepared fresh daily. After MAP stabilized, we injected PBG (40 µg/kg) into the descending thoracic aorta while recording MAP. Tropisetron (200 µg/kg) was injected intravenously 15 min after the initial administration of PBG. We repeated administration of
PBG (40 µg/kg ia) 15 min after treatment with tropisetron (i.e., 30 min after the initial injection of PBG).

Celiac and superior mesenteric ganglionectomy. To examine the extent to which the rise in MAP was attributable to the reflex pressor response evoked by PBG through stimulation of abdominal visceral afferents, four animals were studied before and after ganglionectomy. PBG (40 µg/kg) was injected into the thoracic descending aorta, and the blood pressure response was recorded. The celiac and superior mesenteric ganglia then were isolated and removed, and the animal was rechallenged with PBG (40 µg/kg) 30 min after the initial intra-arterial injection of PBG. Lack of a pressor response to bradykinin (5–10 µg/ml) applied to the gallbladder was used as the criterion for successful ganglionectomy.

Response of abdominal sympathetic afferents to PBG. In eight open-chest cats, we examined the effect of PBG on the discharge activity of ischemically sensitive sympathetic C fiber afferents. After identification of an ischemically sensitive unit with a receptive field in the abdominal region, we injected 40 µg/kg of PBG into the thoracic descending aorta or into the femoral vein while recording afferent activity and MAP. At least 30 min for recovery between injections was maintained.

In seven separate animals, using a similar protocol, we also examined the response of ischemically insensitive C fiber afferents to PBG. After identification of an ischemically insensitive C fiber, we injected 40 µg/kg of PBG into the thoracic descending aorta while recording afferent activity. If the afferent did not respond to PBG, bradykinin (10 µg) was injected intra-arterially to document that it was accessible. We have demonstrated previously that this concentration of bradykinin stimulates most ischemically insensitive C fiber afferents (29).

Additionally, in six other cats, we examined the response of Aδ fiber afferents to PBG using a similar protocol. After identification of an Aδ fiber, we injected 40 µg/kg of PBG into the thoracic descending aorta while recording afferent activity. If the afferent did not respond to PBG, the receptive field was manipulated mechanically to establish that the nerve ending was viable.

Effect of 5-HT3 receptor blockade on response of afferents to PBG. In seven open-chest cats, we examined the effect of 5-HT3 receptor blockade with tropisetron on the response of ischemically sensitive abdominal sympathetic C fibers to PBG. After identification of an ischemically sensitive unit, we injected PBG (40 µg/kg) into the thoracic aorta while recording afferent activity. We repeated administration of PBG (40 µg/kg ia) 30 min after the initial PBG injection, including at least 15 min after treatment with tropisetron (200 µg/kg iv). After treatment with tropisetron was completed, we injected bradykinin (10 µg) into the descending thoracic aorta to establish responsiveness of the afferent nerve ending.

To differentiate between a drug effect and a time-related variation in afferent response, six additional cats were utilized to determine the repeatability of afferent response to PBG. In this protocol, after identification, each animal was treated identically as noted above except that 0.9% NaCl (2–3 ml iv) was used in place of tropisetron.

Data Analysis

Peak discharge rates of sympathetic afferents were measured over 60 s during 3–5 min of control, and 10 min of ischemia, when the greatest number of spikes occurred (14). We measured the afferent response to PBG by averaging the discharge rate of the afferent during the entire period of response. We assessed the latency of afferent response to ischemia and PBG from the time of arterial occlusion or intra-arterial injection of PBG to the point when sustained discharge activity of afferents exceeded a 50% increase over baseline. If an afferent did not respond to PBG after treatment with tropisetron, an onset latency equal in length to the maximum period of observation was assigned.

Data are expressed as means ± SE. We examined PBG-induced responses of MAP, HR, and afferent discharge activity with a Student’s paired t-test. We used the Wilcoxon signed-rank test to compare data, if data were not normally distributed, as determined by the Kolmogorov-Smirnov test. The effects of repeated injections of PBG and tropisetron and cervical vagotomy and ganglionectomy on the PBG-induced responses of HR, MAP, and the afferents were compared using a one-way repeated-measures analysis of variance with a post hoc Bonferroni t-test. If the data were not normally distributed, they were compared with the Friedman repeated-measures analysis of variance on ranks with the Dunnett’s test. Statistical calculations were performed with SigmaStat (Jandel Scientific Software, San Rafael, CA). Values were considered to be significantly different when P < 0.05.

RESULTS

Responses of MAP and HR to PBG

LV injection of PBG evoked bradycardia and depressor responses in 80% (8 of 10 cats) and a pressor response without a change in HR in 20% (2 of 10 cats). Intra-arterial injection of PBG elicited a tachycardia and pressor responses in all animals, whereas intravenous injection of PBG evoked a bradycardia and depressor responses in all cats. Figure 1 summarizes the effect of the three routes of administration of PBG (40 µg/kg) on MAP and HR in closed-chest animals. MAP was significantly decreased from 109 ± 5 to 90 ± 6 mmHg (n = 8 cats) following LV PBG and increased from 73 to 112 mmHg and from 117 to 188 mmHg in the remaining two cats. We observed significant decreases in MAP from 103 ± 5 to 69 ± 6 mmHg following intravenous PBG (n = 10 cats). In contrast, MAP was increased significantly from 112 ± 7 to 138 ± 9 mmHg (n = 10 cats) following intra-arterial administration of PBG into the thoracic aorta (Fig. 1A). We found marked decreases in HR from 208 ± 10 to 153 ± 11 and 193 ± 10 to 126 ± 10 beats/min following LV and intravenous PBG, respectively, compared with increases in HR from 194 ± 10 to 203 ± 11 beats/min following intra-arterial injection of PBG (Fig. 1B). Injection of the same volume of normal saline into any of the three locations did not alter MAP or HR.

Response of MAP and HR to LV PBG Following Denervation

Figure 2 shows representative recordings from one cat in each group. An abrupt bradycardia was noted when the vagal and sympathetic afferent pathways were intact (Fig. 2, A and D). Hypotension coincided with the bradycardia. The response to LV PBG after bilateral cervical vagotomy (Fig. 2B) consisted of a slight tachycardia and marked hypertension. Similar responses were recorded after subsequent celiac and superior mesenteric ganglionectomy (Fig. 2C). In another cat, we observed an abrupt bradycardia and
hypotension in response to PBG following celiac and superior mesenteric ganglionectomy (Fig. 2E). This response was converted to marked hypertension and slight tachycardia in response to PBG after subsequent bilateral cervical vagotomy (Fig. 2F). Figures 3 and 4 (B and C) summarize the MAP and HR data in response to PBG in these two groups. In both groups of cats, application of bradykinin (10 µg/ml) to the gallbladder evoked a pressor response (increase in MAP from 124 ± 6 to 174 ± 7 mmHg; P < 0.05), a response that was abolished by removal of celiac and superior mesenteric ganglia (103 ± 6 to 104 ± 7 mmHg). This alteration in the magnitude of change of MAP and HR to LV administration of PBG was not the result of a time effect or surgical manipulation, because we observed consistent responses of MAP and HR to three repeated injections of PBG into the LV (Figs. 3A and 4A). Additionally, baseline MAP and HR were insignificantly increased by vagotomy (n = 4 cats) (116 ± 8 to 145 ± 14 mmHg, 195 ± 16 to 204 ± 9 beats/min, respectively). Conversely, baseline MAP was insignificantly decreased (130 ± 6 to 110 ± 9 mmHg) and HR was insignificantly increased (184 ± 23 to 200 ± 23 beats/min) by celiac and superior mesenteric ganglionectomy in four other cats.

Dose-Response Studies

Table 1 summarizes the dose-response data obtained following intra-arterial and intravenous administration of PBG (10–100 µg/kg) in five cats. The lowest dose of PBG studied (10 µg/kg ia) significantly increased MAP by 24 ± 8 mmHg. The highest dose of PBG (100 µg/kg ia) significantly increased MAP by 50 ± 11 mmHg. In contrast, depressor responses were noted following injection of PBG (10–100 µg/kg) into the femoral vein. The same volume of normal saline injected either intra-arterially or intravenously did not alter arterial blood pressure.

Reproducibility Studies

We observed consistent pressor responses following two consecutive intra-arterial administrations of PBG. Figure 5, A and B, shows original recordings of MAP from one cat in this group and documents a repeatable increase in blood pressure. Absence of tachyphylaxis following a 30-min period between successive injections of PBG (40 µg/kg ia) was confirmed in seven animals by demonstrating similar increases in MAP (43 ± 5 vs. 42 ± 5 mmHg; P > 0.05) comparing the first to the second applications of PBG (Fig. 6A). Subsequent studies therefore used a 30-min period between successive intra-arterial injections of PBG (40 µg/kg).

Effect of 5-HT3 Receptor Blockade on Response of MAP to PBG

Blockade of 5-HT3 receptors with tropisetron was examined in six animals. Tropisetron (200 µg/kg iv) did not alter the baseline MAP (86 ± 6 before vs. 84 ± 6 mmHg after treatment) but abolished the pressor response to intra-arterial PBG (40 µg/kg) (Fig. 6B).

Pressor Response to PBG After Ganglionectomy

The intra-arterial PBG-induced changes in MAP were reduced 68% by removal of the celiac and superior mesenteric ganglia in four cats. Thus intra-arterial injection of PBG (40 µg/kg) increased MAP by 45 ± 7 mmHg before and 15 ± 5 mmHg after (P < 0.05) ganglionectomy. Figure 5, C and D, shows original recordings of arterial blood pressure from one cat in this group, which shows the effect of ganglionectomy on the pressor response to intra-arterial PBG. Ganglionectomy also abolished the bradykinin-induced increase in MAP (70 ± 17 before vs. 6 ± 4 mmHg after; P < 0.05).

Response of Abdominal Sympathetic Afferents to PBG

Ischemically sensitive sympathetic C fiber afferents. Representative tracings of an ischemically sensitive C fiber innervating the pancreas with a CV of 0.64 m/s are shown in Fig. 7. Intra-arterial but not intravenous injection of PBG stimulated this ischemically sensitive C fiber afferent after an onset latency of 1 s. Figure 8A shows the effect of ischemia, intra-arterial, and intravenous...
nous application of PBG (40 µg/kg) on the discharge activity of 10 ischemically sensitive C fiber afferents (CV = 0.72 ± 0.08 m/s). These nerve endings were located in the mesentery, pancreas, porta hepatis, bile duct, or gallbladder (Table 2). Inflation of the aortic occlusion cuff significantly decreased MAP from 91 ± 12 to 13 ± 2 mmHg (P < 0.05). We have shown previously that this degree of arterial occlusion is associated with a significant increase in portal venous blood and mesenteric lymph lactate concentration within 5 min (22, 23) and a significant decrease in portal venous blood and tissue PO2 (14). A 10-min period of ischemia significantly increased the discharge activity of 10 C fibers from 0.02 ± 0.01 to 1.01 ± 0.03 impulses/s after an onset latency of 245 ± 45 s.

Injection of PBG into the descending thoracic aorta stimulated all 10 C fiber afferents, increasing their discharge activity from 0.12 ± 0.03 to 0.99 ± 0.15 impulses/s (P < 0.05), after an average onset latency of 2.6 ± 0.4 s, and resulting in an increased MAP from 81 ± 4 to 113 ± 6 mmHg (P < 0.05). In contrast, intravenous injection of the same dose of PBG did not stimulate any of these afferents (0.08 ± 0.05 vs. 0.07 ± 0.04 impulses/s; P > 0.05), although MAP was reduced from 82 ± 5 to 55 ± 3 mmHg (P < 0.05).

Ischemically insensitive sympathetic C fiber and Aδ fiber afferents. Figure 8B summarizes the responses of 10 ischemically insensitive C fiber afferents during ischemia, intra-arterial PBG, and bradykinin. These nerve endings were located in the mesentery, pancreas, porta hepatis, gallbladder, and duodenum (n = 5). Ischemia did not alter the discharge activity of any of these 10 C fibers. The average CV (0.89 ± 0.08 m/s) of these fibers was not significantly different from the values for ischemically sensitive fibers. We observed that intra-arterial PBG (40 µg/kg) did not stimulate any of these afferents, whereas intra-arterial bradykinin stimulated 8 of 10 afferents (0.02 ± 0.02 vs. 1.83 ± 0.46 impulses/s; P < 0.05). In addition, we found that intra-arterial PBG (40 µg/kg) did not stimulate any of the eight Aδ fibers (CV = 7.3 ± 1.8 m/s). These nerve endings were located in the pancreas (n = 2), porta hepatis (n = 3), mesentery (n = 1), and bile duct (n = 2). Each Aδ fiber afferent responded to mechanical manipulation.

Fig. 2. Representative tracings from 2 cats following LV injection of PBG before and after denervation. Arrows: PBG (40 µg/kg) was injected as a bolus. Top rows show responses of MAP and HR to PBG before (vagal and sympathetic afferents intact, A), after bilateral cervical vagotomy (B), and after bilateral vagotomy plus celiac and superior mesenteric ganglionectomy (C) in a cat. Bottom rows demonstrate responses of MAP and HR to PBG before (vagal and sympathetic afferents intact, D), after celiac and superior mesenteric ganglionectomy (E), and after celiac and superior mesenteric ganglionectomy plus bilateral cervical vagotomy (F) in a second cat. bpm, Beats per minute.
gallbladder (Table 2). Tropisetron (200 µg/kg iv) did not alter MAP (87 ± 8 before vs. 89 ± 10 mmHg after treatment) but virtually eliminated the responses of the afferent to PBG (Fig. 9B). This reduced responsiveness of afferents to PBG was not due to a generalized decrease in reactivity over time, because six other ischemically sensitive abdominal sympathetic afferents (CV = 0.56 ± 0.07 m/s) responded consistently to repeated injection of PBG (40 µg/kg) over the same time frame (Fig. 9A). These nerve endings were located in the pancreas, porta hepatitis, bile duct, and gallbladder (Table 2). Also, each of the seven afferents still responded to bradykinin (10 µg ia, 0.05 ± 0.02 to 1.32 ± 0.22 impulses/s, P < 0.05) after tropisetron.

**DISCUSSION**

Three important observations were made in this study. First, PBG injected into the LV most commonly evoked depressor and bradycardia responses when vagal and sympathetic afferents were intact. After bilateral cervical vagotomy, PBG administered into the LV evoked a pressor response, that, for the most part, originated outside the mesenteric region. Second, intra-arterial PBG, administrated into the descending thoracic aorta, consistently elicited a pressor response, in part, through activation of 5-HT3 receptors present in the mesenteric region. We observed that injection of PBG into the descending thoracic aorta induced a dose-dependent increase in MAP, whereas intravenous injection of PBG caused bradycardia and dose-dependent depressor responses. Third, intra-arterial PBG
caused a reflex pressor response, in part, by selective activation of ischemically sensitive abdominal sympathetic afferents through a 5-HT₃ receptor mechanism. We found that intra-arterial, but not intravenous, PBG selectively stimulates abdominal ischemically sensitive rather than ischemically insensitive sympathetic afferents to evoke this pressor response. Blockade of 5-HT₃ receptors with tropisetron abolished the pressor as well as the sympathetic afferent responses to intra-arterial PBG. A small part of the pressor response (32%) induced by intra-arterial PBG originates outside the territory of the splanchnic nerve, because celiac and superior mesenteric ganglionectomy markedly attenuated but did not fully eliminate this effect.

Table 1. Changes in mean arterial pressure after injection of PBG

<table>
<thead>
<tr>
<th>Dose of PBG, µg/kg</th>
<th>10</th>
<th>20</th>
<th>40</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Response</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-arterial</td>
<td>105±5</td>
<td>24±8*</td>
<td>96±7</td>
<td>108±3</td>
</tr>
<tr>
<td>Intravenous</td>
<td>92±6</td>
<td>−10±5</td>
<td>96±7</td>
<td>−14±9*</td>
</tr>
</tbody>
</table>

Values reflect means ± SE; units are mmHg; n = 5 cats. PBG, phenylbiguanide. Control, absolute blood pressure; Response, changes in blood pressure. *Significantly different from control, P < 0.05.

Fig. 5. Representative chart recordings of responses of arterial blood pressure to intra-arterial injection of PBG. Arrows: PBG (40 µg/kg) was injected as a bolus. Responses of arterial blood pressure to initial (A) and repeated (B) injection of PBG without ganglionectomy as well as responses of blood pressure to PBG before (C) and after (D) celiac and superior mesenteric ganglionectomy are shown.
afferents. In contrast to previous investigations in which intravenous or right atrial injection of PBG evoked neither reflex hypotension nor hypertension after cervical vagotomy (33–35), we found that LV PBG consistently produced an increase in blood pressure after the procedure of bilateral cervical vagotomy. In aggregate, our data suggest that PBG administered into the left side of the circulation stimulates sympathetic afferents that can lead to a reflex pressor response. It is necessary, however, to cut the vagus nerves to consistently demonstrate the sympathetic afferent response when PBG is injected into the LV. This finding is similar to our observation with right-sided (venous) injection of PBG in which we failed to observe activation of splanchnic afferents. Also, Dawes and Mott (7) observed that intravenous injection of PBG (2–5 mg) can cause a rise in blood pressure after bilateral vagotomy, a finding that is consistent with our observations. Second, the species used in the majority of the other studies were rats (33–35) or rabbits (9, 21). Thus species differences from the cat may be responsible for the absence of a pressor response to LV administration of PBG. We believe, therefore, that our results are not at variance with previous studies when one takes into account the route of injection or the different species studied.

Sympathetic afferents innervate the abdominal region, and several investigations have shown that stimulation of these afferents by ischemia, chemicals, or electrically reflexly increases arterial blood pressure (25, 27, 30). From our data, circulation of PBG downstream from the heart stimulates sympathetic afferent nerve endings in this region. We chose to investigate the effect of intra-arterial rather than LV PBG, because the latter route of administration predominantly evokes depressor and bradycardia responses by stimulating vagal afferent endings. Evans and co-workers (9) have observed that injection of PBG into the proximal aorta increases arterial blood pressure, although they did not evaluate the origin of this response. Interestingly, we found that injection of PBG into the descending thoracic aorta increases arterial blood pressure, although they did not evaluate the origin of this response. Interestingly, we found that injection of PBG into the descending thoracic aorta, but not into femoral vein, specifically stimulates abdominal ischemically sensitive rather than ischemically insensitive sympathetic afferent nerve endings that significantly contribute to the reflex pressor response. We also observed that Aδ fibers were not activated by PBG. The pressor response was reduced by more than two-thirds following denervation of the afferent pathway from the upper abdominal region. These data indicate that intra-arterial PBG evokes a reflex pressor response mainly through activation of abdominal ischemically sensitive sympathetic C fiber afferents.

Evidence from previous studies in our laboratory and others has documented that 5-HT3 receptors are located on both vagal and sympathetic afferents (9, 12, 17). For instance, Grundy et al. (15) observed that 5-HT stimulates vagal afferents innervating the mucosal region through activation of 5-HT3 receptors. We have shown that endogenous 5-HT is released and stimulates ischemically sensitive abdominal sympathetic visceral afferents through a 5-HT3 receptor mechanism.
Pharmacological studies also have demonstrated that PBG exerts its action through 5-HT₃ receptors (15, 19). In this regard, it has been determined that the effects of PBG on the rat vagus nerve can be blocked in vitro with a specific 5-HT₃ receptor antagonist (11, 19). Evans et al. (9) found that injection of PBG into the left atrium, right atrium, or pulmonary artery of unanesthetized rabbits causes a dose-dependent fall in HR and MAP, effects that are mediated by 5-HT₃ receptors present on cardiac and pulmonary vagal afferents. Ireland and Tyers (19) also have found that PBG mimics the depolarizing action of 5-HT on the isolated rat cervical vagus nerve through activation of 5-HT₃ receptors. In addition, Blair et al. (4) have observed that the excitatory effects of PBG on spinal neurons are mediated by 5-HT₃ receptors on cardiac sympathetic afferents. From this information, we proposed that the action of PBG on pressor response and activity of sympathetic afferents are mediated by 5-HT₃ receptors. We have found that blockade of 5-HT₃ receptors with tropisetron, a selective 5-HT₃ receptor antagonist, not only abolished the pressor response but also eliminated the responses of the afferents to intravenous PBG. These data indicate that LV PBG mainly evokes bradycardia and depressor responses when vagal and sympathetic afferents are intact, it can, on occasion, cause a pressor response and consistently elicits a tachycardia and increased blood pressure after bilateral cervical vagotomy. These data indicate that LV PBG activates not only vagal afferents but also sympathetic afferents innervating the heart and lungs and more distal regions. In this latter regard, our data demonstrate that injection of PBG into the descending thoracic aorta causes reflex pressor responses mostly associated with the selective stimulation of nerve endings of ischemically sensitive abdominal sympathetic C fiber afferents. One must be careful, therefore, in central neuronal studies in which PBG is administered into the left side of circulation, because this 5-HT₃ receptor agonist is capable of stimulating both vagal and sympathetic afferent nerve endings. Clearly, it cannot be regarded as a selective stimulator of any one group of afferents.

In conclusion, PBG, through a 5-HT₃ receptor mechanism, produces differential hemodynamic effects according to the site of its administration. When PBG is injected either intravenously or into the LV, it stimulates cardiopulmonary vagal afferents leading to a...
predominant bradycardia and depressor response. However, administration of PBG into the LV or thoracic aorta also activates sympathetic afferents distal to the heart, including selective stimulation of abdominal ischemically sensitive afferents, which contribute significantly to the reflex increase HR and blood pressure. Thus parenteral PBG leads to distinctive hemodynamic responses consisting of either reflex activation or depression of the cardiovascular system. It is important for investigators to be aware of the diversity of these responses mediated by PBG, because afferents encode information in a differential manner from anatomically distinct visceral regions to cardiovascular centers of the brain stem. Thus the response to PBG varies according to the location of administration.

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Address for reprint requests: L.-W. Fu, Division of Cardiovascular Medicine, TB 172, Univ. of California, Davis, Davis, CA 95616.

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### Table 2. Location of abdominal ischemically sensitive and insensitive sympathetic C fiber afferent endings

<table>
<thead>
<tr>
<th>Location</th>
<th>Ischemically Sensitive</th>
<th>Ischemically Insensitive</th>
<th>PBG ia + PBG iv</th>
<th>Repeat PBG</th>
<th>Trop + PBG</th>
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Values reflect numbers of afferent endings. Trop, Tropisetron; ia, intra-arterial; iv, intravenous.
REFERENCES


