Hydrogen Peroxide–Induced Cardiovascular Reflexes
Role of Hydroxyl Radicals

Gregory L. Stahl, Barry Halliwell, and John C. Longhurst

Mesenteric ischemia reflexly activates the cardiovascular system. In addition, mesenteric ischemia and reperfusion generate reactive oxygen species. However, the ability of these short-lived reactive oxygen species to generate cardiovascular reflexes is unknown. We therefore investigated cardiovascular reflexes induced by serosal application of hydrogen peroxide (H₂O₂) to the gallbladder, stomach, or duodenum in anesthetized cats. Serosal application of hydrogen peroxide (44 μmol) to the gallbladder (n=14) significantly (p<0.05) increased mean arterial blood pressure (MAP) by 37±6 mm Hg, left ventricular dP/dt by 1,893±416 mm Hg/sec, heart rate by 6±1 beats per minute, and systemic vascular resistance from 0.34±0.01 to 0.42±0.04 peripheral resistance units. The cardiovascular effects were dose-dependent over a range of 0.4 pmol to 132 μmol H₂O₂. Celiac and superior mesenteric ganglionection abolished H₂O₂-induced cardiovascular effects. Dimethylthiourea (10 mg/kg), a reactive oxygen species scavenger, significantly (p<0.05) attenuated 44 μmol H₂O₂–induced increases in MAP from 36±3 to 2±2 mm Hg. Deferoxamine (10 mg/kg) also significantly attenuated 44 μmol H₂O₂–induced increases in MAP from 40±7 to 19±10 mm Hg, but iron-loaded deferexamine did not. Aspirin (50 mg/kg) did not attenuate H₂O₂–induced excitation of the cardiovascular system. These data suggest that H₂O₂ activates abdominal visceral afferents to reflexly stimulate the cardiovascular system by a mechanism involving hydroxyl radicals. Thus, reactive oxygen species could modulate systemic vascular tone by stimulating abdominal visceral afferents during mesenteric ischemia and reperfusion. (Circulation Research 1992;71:295–302)

KEY WORDS • afferents • free radical • cat • blood pressure • ischemia • reperfusion

Activation of mechanosensitive and chemosensitive afferents within the abdominal viscera (i.e., gallbladder, stomach, pancreas, and duodenum) induces cardiovascular reflexes.1,2 They are predominantly mediated by Aδ and C fibers that travel with the splanchnic nerve. These reflexes include increased heart rate, blood pressure, myocardial contractility, and total peripheral resistance.

Mesenteric artery occlusion in cats, resulting in ischemia, evokes reflex excitation of the cardiovascular system,3,4 but the mechanisms underlying this effect are poorly understood. Various metabolites produced during ischemia, including bradykinin,1,5–8 lactic acid,9 and prostaglandins,6,10 have been reported to elicit cardiovascular reflexes and to stimulate abdominal sensory endings in the cat.

Periods of visceral ischemia followed by reperfusion are well known to generate free radicals and other reactive oxygen species.5,11,12 The major reactive oxygen species are superoxide radical (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radical (•OH). In the presence of “catalytic” iron ions, O₂⁻ and H₂O₂ interact to form highly damaging species such as •OH.13 Unlike O₂⁻ and •OH, H₂O₂ crosses cell membranes readily.13

Although reactive oxygen species are known to be involved in ischemia-reperfusion injury to the gastrointestinal tract,11 information about their direct action on neuronal function is limited. Colton et al14 observed synaptic depression following application of xanthine and xanthine oxidase mixtures to the squid giant synapse and the lobster neuromuscular junction. H₂O₂ was a major contributor to the effects observed.14 A preliminary report from Rozsa and Leung15 demonstrated that intraduodenal administration of very large concentrations of H₂O₂ (7 or 14 M) induced biphasic changes in blood pressure and intestinal blood flow in the rat that were inhibited by hexamethonium and guanethidine. Thus, some evidence suggests that reactive oxygen species may modulate neuronal function. Since the endings of abdominal visceral afferents are more closely associated with the serosal than the mucosal lining of abdominal viscera,16 we decided to investigate the cardiovascular response to serosal application of H₂O₂ to abdominal visceral organs in the anesthetized cat. H₂O₂ was selected because it is known to be generated during ischemia and reperfusion and to be capable of crossing cell membranes.13 To achieve localized application of H₂O₂ to a specific organ, we applied very small amounts

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of concentrated H$_2$O$_2$ solutions directly to the tissues in question. Our results show that H$_2$O$_2$ induces reflex excitation of the cardiovascular system, and that this effect may be mediated by hydroxyl radicals.

**Materials and Methods**

**Surgical Preparation**

Studies were performed on adult cats of either sex (2.5–5.2 kg). Anesthesia was induced by injection of ketamine (20–30 mg/kg i.m.) followed by bolus intravenous injection of α-chloralose (50–80 mg/kg). Additional injections of α-chloralose (5–10 mg/kg i.v.) were given as needed to maintain an adequate depth of anesthesia. The trachea was intubated, and respiration was maintained artificially (Harvard pump, model 661, Ealing, South Natick, Mass.). The right femoral vein was cannulated for administration of drugs and fluids. Arterial blood pressure was monitored by a pressure transducer (Statham P23ID) attached to a cannula inserted in the right femoral artery. Left ventricular pressure was measured with a high-fidelity, catheter-tip pressure transducer (Millar PC-350) placed in the left ventricle through the left carotid artery. Left ventricular dP/dt was obtained by processing the left ventricular pressure signal with a differentiator amplifier. In addition, LV dP/dt at a developed pressure of 40 mm Hg was calculated as an index of myocardial contractility thought to be affected minimally by alterations in pre- or afterload. Systemic vascular resistance was calculated as the ratio of mean arterial pressure (MAP) to mean aortic flow. Heart rate was measured with a cardiotachometer triggered by the left ventricular dP/dt signal. A ventral midline incision exposed the abdominal viscera. Subsequently, the gallbladder, stomach, and proximal duodenum were positioned so that they were isolated from the rest of the abdominal organs. In six cats, a midline sternotomy was performed and an electromagnetic flow transducer was positioned on the ascending aorta. All measurements were recorded continuously on an electrostatic recorder (model ES1000, Gould, Cleveland, Ohio).

Care was taken to ensure that the exposed viscera were moistened adequately with saline solution at 37°C throughout the experiment. Arterial blood gases and pH were measured periodically in all animals with a blood gas analyzer (Radiometer ABL-3). These variables were kept within normal limits by enriching the inspired O$_2$ supply and correcting the arterial pH by infusion of 8% sodium bicarbonate as necessary.

**Experimental Protocols**

**Dose–response studies.** In 18 animals, three applications of various concentrations (2.9–290 mM) of H$_2$O$_2$ (Sigma) were painted on the serosal surface of the gallbladder with a cotton-tipped swab as described previously. This method of application distributes approximately 50 μl of fluid with each application. Thus, in our study three applications of 290 mM H$_2$O$_2$ applied a total of 44 μmol of H$_2$O$_2$. Therefore, three applications of 880 mM, 290 mM, 29 mM, 2.9 mM, 0.29 mM, or 2.9 μM H$_2$O$_2$ resulted in 130 μmol, 44 μmol, 4.4 μmol, 44 nmol, or 0.4 nmol of H$_2$O$_2$ added to the serosal surface, respectively. The order of application was randomized, with at least two different concentrations applied to any one cat. After the maximal hemodynamic response, the gallbladder was washed with at least 10 ml of warm saline that subsequently was suctioned from the abdominal cavity. The dose–response curve was generated with various doses of H$_2$O$_2$ applied at least 30 minutes apart to avoid tachyphyaxis. In several animals, the cardiovascular response to H$_2$O$_2$ application to the stomach and duodenum was examined. Onset latency of the cardiovascular response was described as the time needed to increase systolic left ventricular pressure (LVP) by 10 mmHg after the first application of H$_2$O$_2$. Concentrations of H$_2$O$_2$ were made fresh daily by diluting 30% H$_2$O$_2$ in normal saline.

**Reproducibility studies.** In seven animals, H$_2$O$_2$ (290 mM) was applied to the gallbladder while hemodynamic parameters were monitored. The animal was rechallenged with H$_2$O$_2$ 30 minutes after the initial application of H$_2$O$_2$.

**Ganglionectomy studies.** In four animals, H$_2$O$_2$ (290 mM) was applied to the gallbladder while hemodynamic parameters were monitored. The superior mesentery and celiac ganglia were then isolated and removed. The animal was rechallenged with H$_2$O$_2$ 30 minutes after the initial application of H$_2$O$_2$. Serosal application of bradykinin (1 μg/ml) was then applied to the lower intestinal tract or colon to demonstrate a reflexogenic preparation following ganglionectomy.

**Dimethylthiourea studies.** In five animals, H$_2$O$_2$ (290 mM) was applied to the gallbladder while hemodynamic parameters were monitored. The effects of a free radical scavenger on the cardiovascular effects of H$_2$O$_2$ were evaluated after administration of 10 mg/kg dimethylthiourea (DMTU) (Sigma). DMTU was dissolved in 10 ml of normal saline and was given intravenously over 10–15 minutes. The animal was rechallenged with H$_2$O$_2$ 10 minutes after completion of infusion and at least 30 minutes after the initial application of H$_2$O$_2$. Bradykinin (1 μg/ml) was then applied to the serosal surface of the lower intestinal tract or colon to demonstrate that the preparation was still reflexogenic.

**Deferoxamine studies.** In five animals, H$_2$O$_2$ (290 mM) was applied to the gallbladder while hemodynamic parameters were monitored. The effects of iron chelation on H$_2$O$_2$-induced cardiovascular effects were evaluated after intravenous administration of 10 mg/kg deferoxamine (DEF) (Sigma). DEF was dissolved in normal saline and infused over 30 minutes. The animal was rechallenged with H$_2$O$_2$ 5–10 minutes after completion of DEF infusion and at least 30 minutes after the initial application of H$_2$O$_2$. Bradykinin (1 μg/ml) was then applied to the serosal surface of the lower intestinal tract or colon to demonstrate that the preparation was still reflexogenic.

A control group of four animals received iron-loaded DEF as described by Hatherill et al. Briefly, iron-saturated DEF was prepared by adding 98 mg FeCl$_3$·6H$_2$O to 1 ml DEF (250 mg/ml) for 1 hour at room temperature. After the initial response to H$_2$O$_2$, iron-loaded DEF (10 mg/kg) was infused over 30 minutes. The animal was rechallenged with H$_2$O$_2$ 5–10 minutes after completion of the iron-loaded DEF infusion.

**Aspirin studies.** In six animals, H$_2$O$_2$ (290 mM) was applied to the gallbladder while hemodynamic parameters were monitored. The effects of cyclooxygenase
blockade on H\textsubscript{2}O\textsubscript{2}-induced cardiovascular effects were evaluated after intravenous administration of 50 mg/kg acetylsalicylic acid (ASA) (Sigma). ASA was dissolved in 1N NaOH, diluted in normal saline, and given intravenously over 10 minutes as we have described previously.\textsuperscript{10} This concentration of ASA effectively blocks cyclooxygenase in cats.\textsuperscript{10} The animal was rechallenged with H\textsubscript{2}O\textsubscript{2} 30 minutes after completion of ASA infusion.

Data analysis. Data are presented as mean\(\pm\)SEM. The control and maximal H\textsubscript{2}O\textsubscript{2}-induced responses were collected and averaged over four to five complete cardiac cycles. The maximal H\textsubscript{2}O\textsubscript{2}-induced responses were compared with control values, taken before and after pharmacological intervention, by Student’s \(t\) test for paired data. Differences were considered to be statistically significant at \(p<0.05\).

Results

Dose–Response Studies

Serosal application of H\textsubscript{2}O\textsubscript{2} to the stomach, gallbladder, or duodenum evoked a cardiovascular response. Figure 1 is a tracing from one cat that received four different concentrations of H\textsubscript{2}O\textsubscript{2} to the gallbladder. Each H\textsubscript{2}O\textsubscript{2} application was separated by at least 30 minutes with the exception of the lowest concentration (i.e., 0.3 mM), which in this preparation did not evoke a cardiovascular response. We observed dose-dependent increases in LVP, MAP, and LV dP/dt.

Figure 2 summarizes the dose–response data obtained following serosal application of H\textsubscript{2}O\textsubscript{2} to the gallbladder. The lowest dose of H\textsubscript{2}O\textsubscript{2} studied (i.e., 2.9 \(\mu\)M) increased LVP, MAP, and LV dP/dt in only one of seven cats studied. H\textsubscript{2}O\textsubscript{2} (290 mM) significantly increased MAP from 108±6 to 148±9 mm Hg, heart rate from 170±7 to 177±7 beats per minute, systolic LVP from 140±6 to 185±10 mm Hg, LV dP/dt from 5,779±492 to 7,871±789 mm Hg/sec, and LV dP/dt at a developed pressure of 40 mm Hg from 4,057±574 to 4,657±582 mm Hg/sec. The onset latency was 19±5 seconds and the time to peak response was 46±5 seconds after application of 290 mM H\textsubscript{2}O\textsubscript{2} to the gallbladder.

In six open-chest cats, aortic blood flow was unchanged following application of 290 mM H\textsubscript{2}O\textsubscript{2} to the gallbladder (control, 398±28 ml/min; H\textsubscript{2}O\textsubscript{2}, 396±29 ml/min; \(p>0.05\)), despite significant increases in MAP from 106±4 to 135±6 mm Hg, systolic LVP from 127±7 to 155±9 mm Hg, and LV dP/dt from 4,550±384 to 6,050±931 mm Hg/sec. Thus, these cats demonstrated a significant (\(p<0.05\)) increase in calculated total peripheral resistance from 0.34±0.01 to 0.42±0.04 peripheral resistance units.

Cardiovascular effects also were observed when H\textsubscript{2}O\textsubscript{2} was applied to the stomach and duodenum. Figure 3 summarizes the data obtained from seven cats following application of H\textsubscript{2}O\textsubscript{2} (290 mM) to the gallbladder or stomach. Serosal application of H\textsubscript{2}O\textsubscript{2} to the stomach or gallbladder significantly increased MAP when applied to either organ. However, a significantly greater increase in MAP was observed after application of H\textsubscript{2}O\textsubscript{2} to the gallbladder. H\textsubscript{2}O\textsubscript{2} (290 mM) application to the duodenum increased systolic LVP by 15±4 mm Hg, MAP by 13±3 mm Hg, and LV dP/dt by 350±65 mm Hg/sec.

Reproducibility Studies

The cardiovascular responses were found to be repeatable following consecutive applications of H\textsubscript{2}O\textsubscript{2}. Absence of tachyphylaxis following a 30-minute period between repetitive applications of H\textsubscript{2}O\textsubscript{2} (290 mM) was confirmed in eight animals by demonstrating a similar
ies therefore used a 30-minute period between successive applications of \( \text{H}_2\text{O}_2 \) (290 mM) to the gallbladder.

**Ganglionection Studies**

\( \text{H}_2\text{O}_2 \)-induced MAP changes were eliminated by removal of the superior mesenteric and celiac ganglia in four cats. Thus, \( \text{H}_2\text{O}_2 \) increased MAP from 109±9 to 155±6 mm Hg \((p<0.05)\) before ganglionection, but did not change MAP after ganglionection. \( \text{H}_2\text{O}_2 \)-induced LV dP/dt and heart rate changes also were inhibited after ganglionection. In contrast, application of bradykinin (1 \( \mu \text{g/ml}, \text{total dose 142 pmol} \)) to the lower intestinal tract or colon increased MAP and systolic LVP, demonstrating that reflex activation of the cardiovascular system was still possible.

**Dimethylthiourea Studies**

Figure 4 is a representative tracing from one cat following application of 290 mM \( \text{H}_2\text{O}_2 \) to the gallbladder before (panel A) and after (panel B) DMTU (10 mg/kg i.v.) treatment. We observed an increase in MAP, systolic LVP, and LV dP/dt after application of \( \text{H}_2\text{O}_2 \) (panel A). Intravenous administration of DMTU did not alter baseline MAP, dP/dt, or systolic LVP. We observed that DMTU inhibited the \( \text{H}_2\text{O}_2 \)-induced cardiovascular effects. In contrast, application of bradykinin (1 \( \mu \text{g/ml}, \text{total dose 142 pmol} \)) to the gallbladder increased MAP, systolic LVP, and LV dP/dt, demonstrating that reflex activation of the cardiovascular system was still possible. In four cats, \( \text{H}_2\text{O}_2 \) increased MAP from 102±8 to 138 mm Hg \((p<0.05)\) and from 104±5 to 106±7 mm Hg (NS) before and after DMTU (10 mg/kg) treatment, respectively. DMTU significantly attenuated \( \text{H}_2\text{O}_2 \)-induced increases in MAP, LV dP/dt, and systolic LVP.

**Deferoxamine Studies**

Figure 5 is a representative tracing from one cat following application of 290 mM \( \text{H}_2\text{O}_2 \) to the gallbladder before (panel A) and after (panel B) DEF (10 mg/kg) treatment. We observed an increase in MAP, systolic LVP, and LV dP/dt after application of \( \text{H}_2\text{O}_2 \) (panel A). Treatment with DEF inhibited the \( \text{H}_2\text{O}_2 \)-induced cardiovascular effects. In contrast, application of bradykinin (1 \( \mu \text{g/ml}, \text{total dose 142 pmol} \)) increased MAP, systolic LVP, and LV dP/dt, demonstrating that reflex activation of the cardiovascular system was still possible.

Figure 6 summarizes the MAP data from five cats before and after treatment with DEF (10 mg/kg, panel A). DEF is not only a chelator of iron ions, but also a free radical scavenger. However, iron-loaded DEF scavenges free radicals equally well, although it cannot bind further iron. DEF significantly \((p<0.05)\) attenuated \( \text{H}_2\text{O}_2 \)-induced increases in MAP (Figure 6), LVP (58±13 versus 21±11 mm Hg, control versus \( \text{H}_2\text{O}_2 + \text{DEF} \)), and LV dP/dt (1,240±322 versus 664±362 mm Hg/sec, control versus \( \text{H}_2\text{O}_2 + \text{DEF} \)). However, iron-loaded DEF (10 mg/kg) did not inhibit \( \text{H}_2\text{O}_2 \)-induced alterations in MAP (Figure 6, panel B), \( \text{H}_2\text{O}_2 \)-induced increases in systolic LVP (45±8 versus 42±6 mm Hg, control versus \( \text{H}_2\text{O}_2 + \text{DEF} + \text{IRON} \)), or LV dP/dt (1,333±393 versus 1,333±288 mm Hg/sec, control versus \( \text{H}_2\text{O}_2 + \text{DEF} + \text{IRON} \)).

**Figure 2.** Line graphs showing changes in mean arterial blood pressure (MAP), left ventricular dP/dt, and heart rate (HR) following serosal application of approximately 150 \( \mu \text{l} \) of various concentrations of \( \text{H}_2\text{O}_2 \) to the gallbladder in anesthetized cats. Closed circles and brackets represent the mean and SEM of five to 14 animals, respectively.

**Figure 3.** Bar graphs showing mean arterial blood pressure (MAP) response following application of \( \text{H}_2\text{O}_2 \) (290 mM) to the stomach or gallbladder in seven cats. * \( p<0.05 \) compared with respective control. ** \( p<0.05 \) change in MAP, gastric vs. gallbladder application of \( \text{H}_2\text{O}_2 \). Bars and brackets represent mean and SEM, respectively.
Aspirin Studies

Table 1 summarizes the effects of cyclooxygenase blockade on H$_2$O$_2$-induced cardiovascular excitation. Cyclooxygenase inhibition was induced by treating the animals with ASA at a dose known to block cyclooxygenase completely. In six cats, H$_2$O$_2$ increased MAP by 38±6 and 36±4 mm Hg (NS), LV dP/dt by 1,250±242 and 1,283±301 mm Hg/sec (NS), and systolic LVP by...
The results of this study provide evidence that serosal application of the reactive oxygen species H$_2$O$_2$ to the stomach, gallbladder, or duodenum results in excitation of the cardiovascular system. Furthermore, the cardiovascular response is the result of reflex activity, since it is abolished by celiac and superior mesenteric ganglionectomy. In this regard, application of H$_2$O$_2$ to the serosal surface of the stomach increased MAP, systolic LVP, heart rate, LV dP/dt, LV dP/dt at 40 mm Hg developed pressure, and systemic vascular resistance. This activation of the cardiovascular system is similar to that observed during stimulation of the gallbladder, pancreas, or stomach with histamine, bradykinin, or capsaicin.

Our findings are consistent with the preliminary data presented by Rozsa and Leung, who showed that intramucosal administration of 0.5 ml H$_2$O$_2$ (7.25 or 14.7 M) decreased intestinal blood flow in the rat. This response was inhibited by superoxide dismutase, guanethidine, or hexamethonium treatment. However, in our study, serosal application of H$_2$O$_2$ stimulated the cardiovascular system at concentrations much lower than those used by Rozsa and Leung. Possibly because of the number of iron-loaded cats was not associated with damage of afferent endings. Celiac and superior mesenteric ganglionectomy abolished H$_2$O$_2$-induced cardiovascular responses. Thus, the afferent fibers associated with H$_2$O$_2$-induced cardiovascular reflex travel in the sympathetic and spinal nerves. These data are consistent with previous studies, in which cardiovascular reflexes elicited by gastric distension or serosal gastric application of histamine or bradykinin are abolished by celiac ganglionectomy.

DMTU inhibited the H$_2$O$_2$-induced cardiovascular reflex. DMTU is a nonspecific scavenger of reactive oxygen species, able to react with H$_2$O$_2$ and hydroxyl free radicals. Thus, these data do not clearly identify the mechanism of the H$_2$O$_2$-induced cardiovascular reflex.

In the presence of transition metals, particularly iron, H$_2$O$_2$ can form reactive radicals such as -OH by the metal-catalyzed Haber-Weiss reaction. Indeed, such reactive radicals are frequently responsible for H$_2$O$_2$-mediated damage. To investigate more thoroughly the mechanism of the H$_2$O$_2$-induced cardiovascular reflex, DEF was administered to bind iron and inhibit -OH formation. DEF was found to inhibit H$_2$O$_2$-induced stimulation of the cardiovascular system. These data suggest that hydroxyl radicals contribute to H$_2$O$_2$-induced reflex excitation of the cardiovascular system. Although DEF has some radical-scavenging properties, the failure of iron-loaded DEF to prevent these reflex responses suggests that iron binding is responsible for its action, since iron-loaded DEF is an equally good radical scavenger.

### Table 1. Effect of Aspirin Treatment on H$_2$O$_2$-Induced Excitation of the Feline Cardiovascular System

<table>
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<tr>
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<th>Before treatment</th>
<th>After treatment</th>
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<tr>
<td></td>
<td>Control</td>
<td>Stimulation</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>110±5</td>
<td>148±10*</td>
</tr>
<tr>
<td>LVP (mm Hg)</td>
<td>129±7</td>
<td>182±14*</td>
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<tr>
<td>LV dP/dt (mm Hg/sec)</td>
<td>4,117±373</td>
<td>5,367±456*</td>
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MAP, mean arterial blood pressure; LVP, systolic left ventricular blood pressure; LV dP/dt, first derivative of left ventricular pressure.

Data represent mean±SEM of six animals. Aspirin (50 mg/kg) did not attenuate H$_2$O$_2$-induced excitation of the cardiovascular system.

*p<0.05 compared with respective control.
We have shown previously that prostaglandins (PGs), particularly PGE$_2$ and PGI$_2$, sensitize abdominal visceral afferents. Further, prostaglandins are necessary for the full development of bradykinin-induced excitation of the cardiovascular system. In addition, H$_2$O$_2$ induces the synthesis of PGE$_2$ and PGI$_2$ from the rat colon. Thus, H$_2$O$_2$-induced excitation of the cardiovascular system may have resulted from H$_2$O$_2$-induced synthesis of endogenously generated prostaglandins. However, ASA did not attenuate H$_2$O$_2$-induced cardiovascular effects. Thus, these data suggest that H$_2$O$_2$-induced excitation of the cardiovascular system is not mediated by endogenously synthesized cyclooxygenase products.

The effect of oxygen-derived free radicals on neuronal function is largely unknown. Colton and colleagues have demonstrated that H$_2$O$_2$ decreased synaptic transmission in the squid giant synapse and the lobster neuromuscular junction. H$_2$O$_2$ also depressed excitatory synaptic transmission in the CA1 region of the hippocampus. However, the interaction of xanthine with xanthine oxidase, which produces superoxide and H$_2$O$_2$, increased the release of L-glutamate, an excitatory neurotransmitter, from the hippocampus.

In our study, H$_2$O$_2$ seemed to excite the abdominal afferent fibers, although additional electrophysiological studies need to be performed for confirmation. Thus, different reactive oxygen species may produce diverse neuronal effects in the central and peripheral nervous systems.

An important consideration arising from these data is whether the concentrations of H$_2$O$_2$ used in this study were in the physiological, pathophysiological, or pharmacological range. Reactive oxygen species are produced following mesenteric ischemia and reperfusion. Nilsson et al. demonstrated that reactive oxygen species are produced after abdominal ischemia and reperfusion at a rate of 0.3–0.6 μmol·min$^{-1}$·100 g intestine$^{-1}$ during the first 30 minutes of reperfusion. However, identification of the specific reactive oxygen species was not possible. Serosal application of only 0.4 μmol H$_2$O$_2$ (i.e., 2.9 mM stock concentration) to the gallbladder stimulated the cardiovascular system (Figures 1 and 2). Given that H$_2$O$_2$ readily enters cells and is rapidly metabolized by catalase and glutathione peroxidase enzymes, it is likely that much of the H$_2$O$_2$ applied to the serosal surface was diluted or degraded considerably by the time H$_2$O$_2$ diffused into the interstitium, reacted with iron to form hydroxyl radicals, and activated the afferent endings. We also observed bubbling after H$_2$O$_2$ application to the organs, which is consistent with catalase activity. Therefore, the concentration of H$_2$O$_2$ arriving at the afferent ending would have been far less than that applied. We also demonstrated that two consecutive applications of H$_2$O$_2$ (290 mM) to the gallbladder yielded similar increases in MAP and LV dp/dt (see “Results”). If this H$_2$O$_2$ concentration resulted in serosal cell injury or death to the sensory nerve ending, one would not expect to observe a similar repeatable excitation of the cardiovascular system. Thus, our data suggest that the potential exists in vivo for free radical–induced cardiovascular reflexes of abdominal origin.

Abdominal ischemia activates abdominal visceral afferents and invokes reflex excitation of the cardiovascular system. Ischemically sensitive abdominal afferents are activated by hypoxia, lactic acid, and prostaglandins. We have shown previously that cyclooxygenase blockade significantly attenuated the increased impulse activity of ischemically sensitive afferents. Reactive oxygen species may also activate these endings during ischemia or hypoxia, since relatively low oxygen tensions are sufficient to generate reactive oxygen species. Further electrophysiological studies on the importance of reactive oxygen species' contribution to the activation of ischemically sensitive abdominal visceral afferents are warranted.

Application of other chemical mediators, such as bradykinin, to the epicardial surface of the dog heart results in pressor, depressor, or variable responses, whereas the cat elicits a pressor response. The reasons for these varying responses in dogs is unknown, but they may be related to the level of anesthesia or dose of bradykinin. It is unknown whether a pressor response would be observed following application of H$_2$O$_2$ to the abdominal viscera in other species.

In conclusion, application of H$_2$O$_2$ to the serosal surface of abdominal viscera in cats dose-dependently invokes reflex excitation of the cardiovascular system. Cardiovascular excitation includes increased systolic LVP, MAP, myocardial contractility, heart rate, and systemic vascular resistance. The afferent pathway passes through the celiac and superior mesenteric ganglia. The H$_2$O$_2$-induced reflex is iron-dependent, suggesting that hydroxyl free radicals or other reactive species mediate part of the reflex.

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