Cardiovascular Reflexes Arising from the Gallbladder of the Cat

Effects of Capsaicin, Bradykinin, and Distension

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SUMMARY. We have studied the cardiovascular responses which can be evoked when the gallbladder is stimulated pharmacologically or mechanically. To determine the potential for reflex cardiovascular activation, we applied capsaicin, a selective thin-fiber agonist, to the serosal surface of the gallbladder. This algesic substance evoked cardiovascular responses which included increases in mean arterial pressure (MAP) by 14%, heart rate (HR) by 3%, left ventricular dP/dt at 40 mm Hg developed pressure (dP/dt DP40) by 14%, and systemic vascular resistance (SVR) by 19%. There were no demonstrable effects on the cardiovascular system when this same substance was applied to the surface of the liver. Bilateral vagotomy at the level of the diaphragm did not alter the responses to capsaicin. Right atrial overdrive pacing did not reduce the positive inotropic effect elicited when the gallbladder was stimulated. Removal of the celiac and superior mesenteric ganglia, or selective denervation of the gallbladder, abolished the cardiovascular responses which were evoked previously. β-Adrenergic blockade, however, abolished only the reflex chronotropic and inotropic responses. Thus, the potential for eliciting reflex cardiovascular alterations by stimulating gallbladder afferents with capsaicin was established. In subsequent studies, stimulating the gallbladder with bradykinin, an endogenous polypeptide, evoked a reflex activation of the cardiovascular system similar to that seen with capsaicin (MAP = 14%; HR = 4%; dP/dt DP40 = 18%; SVR = 14%). These reflex responses were dose dependent, were produced by mucosal as well as serosal application of this substance, and were eliminated by bilateral splanchnic nerve section. In contrast to capsaicin and bradykinin, distension of the gallbladder did not cause any cardiovascular alterations. We conclude that stimulation of gallbladder afferents by the algesic substance capsaicin or by bradykinin, an endogenous substance that under certain conditions may be formed in bile, can induce significant reflex activation of the cardiovascular system (Circ Res 52: 26-35, 1983).

IT is well known that cardiovascular reflexes can be elicited by stimulating receptors in several abdominal organs including the stomach (Guzman et al., 1962; Saphir and Rapaport, 1969; Longhurst et al., 1980), spleen (Guzman et al., 1962; Saphir and Rapaport, 1969; Ferreira et al., 1973; Riccioppo Neto et al., 1974), portal vein (Guzman et al., 1962), and intestine (Saphir and Rapaport, 1962; Frank, 1978). Considerable evidence has also been presented which suggests an interaction between the gallbladder and the cardiovascular system. Epidemiological studies have utilized postmortem data to demonstrate a positive correlation between gallbladder disease and coronary artery disease (Tennant and Zimmerman, 1931; Breyfogle, 1940). Clinical studies have presented evidence that removal of a diseased gallbladder can reduce angina-type pain or electrocardiographic irregularities (Wakefield, 1947; McLemore and Levine, 1955). To establish the reflex nature of this interaction, several investigators have demonstrated that the cardiovascular responses evoked by stimulating the gallbladder are mediated by vagal afferents (Buchbinder, 1930; Scott and Ivy, 1932; Bettman and Rubinfield, 1935; Cullen and Reese, 1952). There is also evidence, however, that sensory information from the gallbladder travels in the splanchnic nerves (Newman, 1974).

What has not been demonstrated by these previous reflex studies, however, is that cardiovascular responses can be evoked by activating afferent fibers from the gallbladder that may transduce the sensation of pain (i.e., group III and group IV afferents). Accordingly, an initial study was undertaken to test the hypothesis that capsaicin, an algesic agent (Toh et al., 1955) known to activate group III and group IV afferent fibers from lung and from skeletal muscle (Colebridge et al., 1964; Kaufman et al., 1981), would reflexly elicit cardiovascular responses when applied to the serosal surface of the gallbladder. However, to demonstrate that the gallbladder reflexly affects the cardiovascular system under physiological or pathophysiological conditions, it is necessary to show that a naturally occurring stimulus can elicit reflex responses. To study this possibility, we proposed and tested two additional hypotheses. The first hypothesis was that physiological or pathophysiological levels of bradykinin, an endogenous polypeptide, would reflexly activate the cardiovascular system when applied to the gallbladder of cats. Bradykinin was chosen as
the excitable agent since this algesic substance, or its precursors, is known to occur throughout the gastrointestinal tract (Amundsen and Nustad, 1965; Zei- tin, 1971, 1972; Frankish and Zeitin, 1980) as well as in bile (Amundsen and Nustad, 1965). The second hypothesis was that mechanical distension of the gallbladder would elicit reflex cardiovascular responses similar to those observed with either capsaicin or bradykinin. A positive response to distension of the gallbladder was expected, since previous studies in dogs and humans had demonstrated cardiovascular responses during distension of or traction on the biliary tract (McArthur and Wakefield, 1945; Hodge and Messer, 1948; Hodge et al., 1974).

Methods

Surgical Preparation

Studies were performed on 48 adult cats of either sex (1.36-4.77 kg). Methoxyflurane anesthesia was induced and maintained (concentration = 0.2%) with a veterinary anesthesia machine and ventilator (Vetaflex 5 and Metomatic, Ohio Medical Products). A femoral vein was cannulated for administering drugs or fluids. Systemic arterial pressure was measured through a femoral artery cannula, which was connected to a pressure transducer (Statham, model P23ID). For those cats in which aortic flow was measured, a median sternotomy was performed to expose the heart. Left ventricular pressure was measured with a micromanipulated catheter pressure transducer (Millar Instruments, model FC-350) which was inserted into the apex of the left ventricle of the heart through a stab incision and secured with a purse-string suture. Left ventricular end-diastolic pressure was obtained by amplifying the left ventricular pressure signal. Left ventricular dp/dt was obtained by processing this same signal with a derivative amplifier (Hewlett-Packard, model 8814A). An electromagnetic flow transducer (Zepeda Instruments) was placed around the ascending aorta to measure blood flow through that vessel. Flow transducers were calibrated in vivo by using a calibrated perfusion pump to pass a known flow of blood through a vessel around which the transducer was placed. Calibration curves were generated over the entire range of flows. For those cats in which aortic flow was not measured, the micromanipulated catheter pressure transducer was inserted into the left ventricle by passing it in a retrograde direction through the carotid artery. Heart rate was measured continuously with a cardiotachometer (Hewlett-Packard, model 8812A) which was triggered by the systemic arterial pressure signal. For certain experiments in which the heart was paced, a bipolar epicardial electrode was sutured to the right atrium. The electrode was then connected to a stimulator and stimulus isolation unit (Grass Instruments, models S4CR and SIU-4A).

All measurements were recorded on an eight-channel thermal stylus recorder (Hewlett-Packard, model 7758B). Additionally, left ventricular dp/dt/dt at a developed pressure of 40 mm Hg was calculated as described previously (Longhurst et al., 1980). This index of myocardial contractility is thought to be minimally affected by alterations in preload or afterload (Mason et al., 1971). Systemic vascular resistance was calculated as the ratio of mean arterial pressure to mean aortic flow, assuming right atrial pressure to be zero.

A midline ventral incision was used to expose the abdominal viscera. Care was taken to be sure that the exposed viscera were moistened adequately with warm Ringer's solution throughout the experiment. Body temperature was monitored continuously (Yellow Springs, model 41TD) and was maintained in a range of 37-39°C by a heating pad and heating lamp. End-tidal CO₂ was measured continuously (Beckman, model LB-2 Gas Analyzer) and was maintained in a range of 4.5-5.0% by adjusting ventilation. Arterial blood pH was monitored frequently (Radiometer, model PHM 62) and was maintained in a range of 7.35-7.45 by administering a 1.5% solution of sodium bicarbonate.

Experimental Protocols

Capsaicin

In 28 cats, capsaicin (200 µg/ml) was applied with a cotton-tipped applicator either to the serosal surface of the gallbladder or to the surface of the liver. It was determined gravimetrically that for each application, 0.05 ml of solution was deposited on the organ. Thus, the total dose of capsaicin applied each time was 10 µg. Capsaicin was dissolved as described previously by Coleridge et al. (1964). For both the gallbladder and the liver, serosal application of the vehicle in which capsaicin was dissolved had no effect on any of the cardiovascular variables that were measured. To reduce the effects of tachyphylaxis to capsaicin, recovery periods of at least 30 minutes were provided after each application. In addition, after the maximal hemodynamic response, the gallbladder was washed with 10 ml of Ringer's solution which subsequently was suctioned gently from the abdominal cavity.

In six cats, an initial and a repeat application of capsaicin were made to the serosal surface of the gallbladder. In five cats, capsaicin was applied to the gallbladder before and after overdrive pacing of the right atrium. In eight cats, capsaicin was applied topically to the gallbladder before and after bilateral vagotomy at the level of the diaphragm. In 12 cats, capsaicin was applied topically to the gallbladder before and after removal of the celiac and superior mesenteric ganglia. In separate experiments in five cats, capsaicin was applied topically to the gallbladder before and after selective denervation of that organ. Following ganglionectionomy or selective denervation, to demonstrate that each animal was capable of reflexly activating the cardiovascular system and had not simply developed tachyphylaxis, capsaicin (200 µg/ml) was applied with a total of 35 cats were studied. In 25 cats, bradykinin (200 µg/ml) was applied to the gallbladder or to the surface of the liver. Bradykinin was dissolved in Ringer's solution and was maintained in a range of 37-39°C by a heating pad and heating lamp. End-tidal CO₂ was measured continuously (Beckman, model LB-2 Gas Analyzer) and was maintained in a range of 4.5-5.0% by adjusting ventilation. Arterial blood pH was monitored frequently (Radiometer, model PHM 62) and was maintained in a range of 7.35-7.45 by administering a 1.5% solution of sodium bicarbonate.

Bradykinin

A total of 35 cats were studied. In 25 cats, bradykinin was applied with a cotton-tipped applicator either to the serosal surface of the gallbladder or to the surface of the liver. Bradykinin was dissolved in Ringer's solution at room temperature to an initial concentration of 1000 µg/ml. Appropriate serial dilutions were made to obtain the desired concentrations. The stock solution of bradykinin was stored in a freezer and was used for no more than 2 weeks before a fresh stock solution was prepared. In general, bradykinin was used at a concentration of 10 µg/ml, since this was the lowest concentration that consistently yielded maximal cardiovascular responses. As with capsaicin, the total amount applied during one stimulation was 0.05 ml. Thus, at a concentration of 10 µg/ml, 500 ng of bradykinin was applied
to the surface of the gallbladder. As a control for the vehicle, it was demonstrated that cold Ringer's solution did not evoke any cardiovascular responses when applied either to the gallbladder or to the liver. To reduce the effects of tachyphylaxis to bradykinin, recovery periods of at least 1 hour were provided after each application. Also, as with capsaicin, after the maximal hemodynamic response, the gallbladder was washed with 10 ml of Ringer's solution.

In nine cats, an initial and a repeat application of bradykinin were made to the serosal surface of the gallbladder. In five cats, bradykinin was applied to the gallbladder before and after overdrive pacing of the right atrium. In six cats, bradykinin was applied to the serosal surface of the gallbladder in a range of concentrations from 10 pg/ml to 100 pg/ml (total doses = 0.5 pg to 5 pg). The order in which the different concentrations were applied was varied randomly. In seven cats, bradykinin was applied topically to the gallbladder before and after bilateral vagotomy at the level of the diaphragm. In nine cats, bradykinin was applied topically to the gallbladder before and after removal of the celiac and superior mesenteric ganglia. In four cats, bradykinin was applied topically to the gallbladder before and after sequential transection of the right and left splanchnic nerves. As with capsaicin, following ganglionectomy or transection of the splanchnic nerves, it was determined that each animal was capable of reflexly activating the cardiovascular system and had not simply developed tachyphylaxis.

In separate experiments in five cats, we compared the cardiovascular responses that were evoked when bradykinin was applied to the serosal surface of the gallbladder to those evoked when this substance was applied to the mucosal surface of that organ. To apply bradykinin to the mucosal surface, the gallbladder was first drained of bile. A small incision then was made, through which the bradykinin could be applied. The amount of bradykinin applied to the mucosal surface was comparable to that which was applied to the serosal surface (0.05 ml). The sequence of surfaces tested was varied randomly.

**Distension of the Gallbladder**

In separate experiments, in seven cats, we distended the gallbladder with pressures up to 100 mm Hg. Two methods were used to distend the gallbladder. In the first, a cannula was inserted into the gallbladder through a small incision and secured with a purse-string suture. Warm Ringer's solution was infused through the cannula to distend the gallbladder. In the second method, a small latex balloon was attached to the end of a cannula which was then inserted into the gallbladder. The balloon and gallbladder then were distended by infusing warm Ringer's solution through the cannula. For both methods, the distending pressure was measured with a transducer (Statham, model P23ID) which was connected to a sideport on the cannula. To demonstrate that stimulation of the gallbladder could evoke reflex cardiovascular responses under these circumstances, bradykinin (10 pg/ml) was applied to the serosal surface of that organ before and/or after distension.

**Statistics**

The control hemodynamic values were compared to the maximal responses by the Student's t-test for paired values. The cardiovascular responses to repetitive application of capsaicin or bradykinin were compared over time by a two-way analysis of variance followed by the Scheffe multiple comparisons procedure to locate significant differences (Glass and Stanley, 1970). In all cases, results were expressed as the mean ± SE and were judged significantly different at P < 0.05.

**Results**

**Responses to Topical Application of Capsaicin or Bradykinin to Gallbladder and Liver**

Capsaicin (200 µg/ml; total dose = 10 µg), applied to the serosal surface of the gallbladder, resulted in a pronounced activation of the cardiovascular system (Figs. 1A and 2). There were significant increases in heart rate, mean arterial pressure, left ventricular systolic pressure, maximal left ventricular dP/dt, and a slight but significant increase in left ventricular end-diastolic pressure. However, aortic flow remained unchanged, which resulted in a significant increase in calculated systemic vascular resistance. Additionally, left ventricular dP/dt at 40 mm Hg developed pressure increased significantly. Each of these cardiovascular variables was altered similarly when bradykinin (10 µg/ml; total dose = 500 ng) was applied to the serosal surface of the gallbladder (Figs. 3A and 4). However, the latency to onset of the responses to bradykinin (13.0 ± 0.9 sec) was significantly longer than that for capsaicin (3.7 ± 0.4 sec). In contrast to the responses evoked when capsaicin or bradykinin was applied to the gallbladder, application of these substances to the serosal surface of the liver failed to elicit a significant change in any of the cardiovascular variables which were measured (Figs. 1B and 3B).

For those cats in which capsaicin or bradykinin was applied repeatedly to the gallbladder, there were no significant differences between the responses evoked by each application. A 30-minute recovery period separated successive applications of capsaicin, whereas applications of bradykinin were separated by a 1-hour recovery period. Following the initial application of capsaicin, heart rate increased from 214 ± 12 to 221 ± 12 beats/min, mean arterial pressure increased from 110 ± 4 to 129 ± 7 mm Hg, and left ventricular dP/dt at 40 mm Hg developed pressure increased from 2854 ± 127 to 3145 ± 153 mm Hg/sec. After the second application of capsaicin, these same variables increased from 210 ± 13 to 218 ± 12 beats/min, from 108 ± 5 to 127 ± 7 mm Hg, and from 2822 ± 181 to 3208 ± 216 mm Hg/sec, respectively. Following the initial application of bradykinin, heart rate increased from 184 ± 9 to 191 ± 9 beats/min, mean arterial pressure increased from 107 ± 6 to 122 ± 7 mm Hg and dP/dt at 40 mm Hg developed pressure increased from 2680 ± 196 to 3167 ± 199 mm Hg/sec. Likewise, following the second application of bradykinin, heart rate increased from 198 ± 9 to 202 ± 9 beats/min, mean arterial pressure increased from 108 ± 4 to 121 ± 3 mm Hg, and dP/dt at 40 mm Hg developed pressure increased from 2867 ± 99 to 3308 ± 164 mm Hg/sec.

In five cats, overdrive pacing of the right atrium failed to alter the increases in maximal left ventricular dP/dt or dP/dt at 40 mm Hg developed pressure that were elicited by topical application of capsaicin or
bradykinin to the gallbladder. When hearts were not paced, application of capsaicin significantly increased heart rate from 207 ± 11 to 214 ± 11 beats/min, whereas maximal left ventricular dP/dt and dP/dt at 40 mm Hg developed pressure increased significantly from 3775 ± 199 to 4389 ± 274 mm Hg/sec and from 2800 ± 140 to 3125 ± 185 mm Hg/sec, respectively. When hearts were paced at 221 ± 8 beats/min, application of capsaicin significantly increased maximal left ventricular dP/dt from 3930 ± 254 to 4738 ± 369 mm Hg/sec and significantly increased dP/dt at 40 mm Hg developed pressure from 2837 ± 222 to 3255 ± 263 mm Hg/sec. Before pacing, bradykinin (10 µg/ml) also evoked significant increases in heart rate (206 ± 11 to 211 ± 11 beats/min), maximal left ventricular dP/dt (3500 ± 215 to 4175 ± 264 mm Hg/sec), and dP/dt at 40 mm Hg developed pressure (2725 ± 257 to 3250 ± 335 mm Hg/sec). During overdrive pacing,
when bradykinin was applied to the gallbladder, there were still significant increases in maximal left ventricular dP/dt (4000 ± 271 to 4850 ± 493 mm Hg/sec) and dP/dt at 40 mm Hg developed pressure (2975 ± 218 to 3450 ± 350 mm Hg/sec).

Figure 5 shows the cardiovascular changes which occurred in one cat when bradykinin (100 μg/ml) was applied to the serosal surface of the gallbladder and was not removed by washing with Ringer's solution. The latency to onset of the pressor response was approximately 13 seconds and the total duration of the response was 5.4 minutes.

**Effects of Selective Denervations on Cardiovascular Responses Evoked by Capsaicin or Bradykinin**

Bilateral vagotomy at the level of the diaphragm did not alter the cardiovascular responses evoked when capsaicin or bradykinin was applied to the gallbladder. For example, as shown for bradykinin in Figure 6A, comparing pre- to post-vagotomy, there were similar significant increases in heart rate, mean arterial pressure, and left ventricular dP/dt at 40 mm Hg developed pressure. In contrast, removal of the celiac and superior mesenteric ganglia abolished the
cardiovascular responses that were evoked previously when capsaicin or bradykinin was applied to the gallbladder (Fig. 6A). In four cats, sequential splanchnic nerve transection just above the diaphragm demonstrated that cutting the right splanchnic nerve and leaving the left splanchnic nerve intact partially re-

![Figure 5. Representative cardiovascular responses evoked by application of bradykinin (BK, 100 μg/ml; total dose = 5 μg) to the gallbladder of a cat.]

![Figure 6. Maximal cardiovascular responses (mean ± SEM) evoked by application of bradykinin (BK, 10 μg/ml; total dose = 0.5 μg) to the gallbladder of cats before and after bilateral vagotomy or celiac and superior mesenteric ganglionectomy (panel A; n = 12), and before and after sequential transection of the right and left splanchnic nerves (panel B; n = 4). C = control, NS = nonsignificant.]

\[ \text{Left Ventricular Pressure (mmHg)} \]
\[ \text{Arterial Pressure (mmHg)} \]
\[ \text{Mean Arterial Pressure (mmHg)} \]
\[ \text{Heart Rate (beats/min)} \]
duced the hemodynamic responses to application of bradykinin to the gallbladder (Fig. 6B). In addition, bilateral splanchnic nerve transection completely abolished the cardiovascular responses evoked when bradykinin was applied to the gallbladder (Fig. 6B). After ganglionecectomy or nerve transection, there were marked increases in arterial blood pressure and heart rate when capsaicin was injected into the femoral artery, thus indicating that the animals were still capable of reflexly activating the cardiovascular system.

Selective denervation of the gallbladder abolished the cardiovascular responses that were evoked previously when capsaicin was applied to the gallbladder. However, following denervation, when capsaicin was injected into the femoral artery, there were still pronounced increases in arterial blood pressure and heart rate.

**Effect of β-Adrenergic Blockade on Cardiovascular Responses Evoked by Capsaicin**

β-Adrenergic blockade abolished the increases in heart rate and left ventricular dP/dt (maximal and at 40 mm Hg developed pressure) that were evoked previously when capsaicin was applied to the gallbladder. Before β-blockade, heart rate increased from 201 ± 14 to 207 ± 13 beats/min and dP/dt at 40 mm Hg developed pressure increased from 2300 ± 146 to 2733 ± 172 mm Hg/sec during stimulation of the gallbladder. However, after β-blockade, heart rate and dP/dt at 40 mm Hg developed pressure were unchanged during stimulation of the gallbladder (156 ± 8 to 156 ± 8 beats/min and 2075 ± 252 to 2075 ± 258 mm Hg/sec). Further, following β-adrenergic blockade, there were still significant increases in mean arterial pressure (105 ± 6 to 128 ± 9 mm Hg), left ventricular systolic pressure (129 ± 12 to 151 ± 13 mm Hg), and left ventricular end-diastolic pressure (6.3 ± 0.7 to 9.1 ± 0.9 mm Hg).

**Bradykinin Dose-Cardiovascular Response Relationship**

There were no cardiovascular responses evoked when 10 pg/ml (total dose = 0.5 pg) of bradykinin was applied to the gallbladder. However, as shown in Figure 7, there were significant increases in mean arterial pressure for concentrations of bradykinin that ranged from 100 pg/ml to 100 μg/ml (total dose = 5 pg to 5 μg). The change in mean arterial pressure in response to the application of bradykinin increased in a sigmoidal fashion in six cats, with the smallest responses occurring at the lower doses and the largest responses occurring at the higher doses. A similar dose-related pattern was also shown for the other cardiovascular variables that were increased when bradykinin was applied to the gallbladder.

**Responses to Application of Bradykinin to Mucosal Surface of Gallbladder**

Bradykinin (10 μg/ml), applied to the mucosal surface of the gallbladder in five cats, evoked small but consistent cardiovascular responses that were directionally similar to those seen when this substance was applied to the serosal surface of the same organ. Thus,
after a latency of 33 ± 4 sec, there were significant increases in mean arterial pressure (84 ± 6 to 97 ± 6 mm Hg), left ventricular dP/dt at 40 mm Hg developed pressure (2138 ± 146 to 2363 ± 122 mm Hg/sec), and calculated systemic vascular resistance (0.26 ± 0.02 to 0.28 ± 0.02 PRU). For these animals, heart rate did not increase significantly (176 ± 14 to 177 ± 16 beats/min).

**Distension of Gallbladder**

Distension of the gallbladder with pressures up to 100 mm Hg failed to evoke a consistent change in any of the cardiovascular variables that were measured. Following distension, to demonstrate that the gallbladder was still capable of activating the cardiovascular system, topical application of bradykinin (10 μg/ml) to the serosal surface continued to elicit significant increases in mean arterial pressure (102 ± 6 to 119 ± 6 mm Hg), left ventricular dP/dt at 40 mm Hg developed pressure (2400 ± 108 to 2650 ± 121 mm Hg/sec), and calculated systemic vascular resistance (0.33 ± 0.05 to 0.38 ± 0.06 PRU). Topical application of bradykinin did not cause pressure within the gallbladder to increase.

**Discussion**

This study demonstrates conclusively for the first time that chemical stimulation of the gallbladder reflexly activates the cardiovascular system. The reflex responses evoked when either capsaicin or bradykinin was applied to the surface of the gallbladder included significant increases in heart rate, mean arterial pressure, left ventricular end-diastolic pressure, maximal left ventricular dP/dt, left ventricular dP/dt at 40 mm Hg developed pressure, and calculated systemic vascular resistance.

Previous studies which investigated the interaction of the gallbladder and the cardiovascular system include those which were epidemiological, and which tended to demonstrate a positive correlation between gallbladder disease and heart disease (Tennant and Zimmerman, 1931; Breyfogle, 1940). Also, clinical studies have demonstrated that various arrhythmias or anginal type pain can be ameliorated by removal of a diseased gallbladder (Wakefield, 1947; McIver and Levine, 1955).

The reflex nature of the interaction between the gallbladder and the heart has been studied in several species (Buchbinder, 1930; Scott and Ivy, 1932; Bettman and Rubinfeld, 1935; Cullen and Reese, 1952). Though the results of these studies have been variable, with activation of the heart in some cases and inhibition in others, the common point of each was that the reflex responses were mediated by vagal afferents. In contrast, the present study demonstrates that chemical stimulation of the gallbladder with capsaicin or bradykinin evokes a profound reflex activation of the cardiovascular system that is unaffected by bilateral vagotomy. The difference between the results of our study and those done previously may reflect either a species difference (cats vs. frogs or dogs) or a difference in the receptors being stimulated and in the subsequent activation of a different afferent pathway.

Stimulation of the gallbladder with capsaicin or bradykinin likely excited nociceptors whose fibers traveled in the splanchnic nerves. In contrast to a previous study which suggested that afferent information from the gallbladder is carried only in the right splanchnic nerve (Newman, 1974), we demonstrated that afferent information from the gallbladder is carried in both splanchnic nerves. In each case, when we cut the right splanchnic nerve alone, the reflex cardiovascular responses were attenuated by approximately 50%. Although not done in the present study, we would expect that the cardiovascular responses would be attenuated similarly if the left splanchnic nerve had been transected first.

Selective denervation of the gallbladder demonstrated that the receptors which give rise to the reflex responses observed are localized to that organ. Denervation abolished the responses that were evoked previously when the gallbladder was stimulated, thus showing that these effects were not the result of an adjacent organ being stimulated or the result of the algesic agent being transported in the blood and exerting its effects elsewhere.

Based on the various latencies to onset of the responses to capsaicin and bradykinin, one might predict that: (1) the receptors stimulated reside in the serosal layer of the gallbladder, and (2) the responses elicited by bradykinin may involve an intermediate substance. The first of these predictions is based on the relative latencies to onset of the responses to bradykinin when applied to the serosal vs. the mucosal surface of the gallbladder (13 ± 1 vs. 33 ± 4 sec). Owing to the thin wall of the gallbladder, the difference in these latencies to onset may reflect the time required for bradykinin to diffuse from the mucosal surface to receptors located closer to the serosal layer. The second prediction is based on the relative latencies to onset of the responses to capsaicin vs bradykinin (4 ± 1 vs. 13 ± 1 sec) when applied to the mucosal surface of the gallbladder. The significantly longer latency to onset for bradykinin may be due to a prostaglandin intermediate which has been shown to be the case when other abdominal visceral organs are stimulated by this endogenous polypeptide (Ferreira et al., 1973). However, both possibilities require additional studies to determine if they are responsible for the differences noted.

The reflex increases in left ventricular dP/dt evoked by the application of capsaicin or bradykinin may have been secondary to: (1) an increased preload, as evidenced by an increased left ventricular end-diastolic pressure, and/or (2) an increased heart rate, i.e., Bowditch effect (Mitchell et al., 1963). That the first of the two possibilities was not the case was demonstrated by the fact that left ventricular dP/dt at 40 mm Hg developed pressure was also increased significantly following topical application of capsaicin or...
bradykinin. Left ventricular dP/dt at 40 mm Hg developed pressure is an index of myocardial contractility that is thought to be relatively independent of changes in filling pressure in the anesthetized preparation (Mason et al., 1971). Also, although statistically significant, the increases in left ventricular end-diastolic pressure that were observed were small and probably had little, if any, effect on maximal left ventricular dP/dt. Additional evidence for this latter point is given by the fact that, although β-adrenergic blockade completely abolished the increases in heart rate and left ventricular dP/dt that were evoked previously in response to capsaicin, there were still significant increases in left ventricular end-diastolic pressure. Further, a primary increase in contractility during stimulation of the gallbladder is supported by the finding that β-adrenergic antagonism abolished the increases in maximal left ventricular dP/dt and dP/dt at 40 mm Hg developed pressure. That the second of the two possibilities, the Bowditch phenomenon, did not have a significant effect on the observed increases in contractility was demonstrated by the fact that right atrial overdrive pacing failed to alter the reflex changes in maximal left ventricular dP/dt or dP/dt at 40 mm Hg developed pressure. Thus, the increase in these indices of myocardial contractility probably was not secondary to these other factors, but was a primary response elicited by the application of capsaicin or bradykinin to the gallbladder.

In the present study, distension of the gallbladder did not evoke any significant cardiovascular responses. This is in contrast to previous studies in man (Hodge and Messer, 1948; McArthur and Wakefield, 1945) and dog (Hodge et al., 1974) in which distension of the gallbladder or biliary tract evoked alterations in the rate, rhythm, or electrocardiographic activity of the heart. Additionally, it has been shown that distension of the gallbladder in the ferret (Cervero, 1982) and the cat (Newman, 1974) produces marked increases in blood pressure. In the latter study, blood pressure increased 50 mm Hg when the gallbladder was distended to an unspecified pressure by means of a balloon. When we used the same technique to distend the gallbladder to a pressure of 100 mm Hg, mean arterial pressure increased only approximately 5 mm Hg. The reason for this discrepancy between the results of our study and those of Newman is uncertain. The threshold pressure observed by Newman may have been greater than 100 mm Hg; however, it is doubtful that pressures within the gallbladder would ever exceed this level. Also, it is doubtful that applying bradykinin to the gallbladder before distension caused a change in the compliance of that organ, altered the degree of stretch per change in pressure, and thus reduced or prevented any reflex cardiovascular responses, since distending the gallbladder before bradykinin was applied also failed to evoke cardiovascular responses.

The present study demonstrates that bradykinin, an endogenous substance that is found in some inflammatory states, stimulates the gallbladder to activate the cardiovascular system reflexly. The significance of this finding is heightened by the further observation that bradykinin can elicit these reflexes in doses that could potentially be produced in rabbit bile (Amundsen and Nustad, 1965). Although rabbit bile can increase its bradykinin activity, it shows little kininase activity (Amundsen and Nustad, 1965). This information suggests that bradykinin formed in bile would not be metabolized rapidly. Thus, under certain conditions associated with an increased production of this substance, such as during inflammation (Lewis, 1970), it is possible that bradykinin in the bile could reach high levels. One inflammatory condition that could be associated with gallbladder and biliary tract inflammation is cholelithiasis. In this regard, it has been demonstrated that some patients with coronary disease experience an increased frequency of angina pectoris in the presence of cholelithiasis (Clark, 1945; Ravdin et al., 1955). Although it is not possible to be certain whether such individuals are experiencing true myocardial ischemia caused by gallbladder-cardiovascular reflexes or are experiencing referred pain from the gallbladder (Miller, 1942; Clark, 1945), it is likely that, at least in some cases, the pain does represent myocardial ischemia. This is supported by evidence that mechanical distension of the gallbladder and biliary tract can produce electrocardiographic T-wave alterations that are consistent with ischemia (McArthur and Wakefield, 1945; Hodge and Messer, 1948). In addition, it has been shown that abnormal T-waves, consistent with a pattern of myocardial ischemia, revert to a normal pattern following cholecystectomy (Hampton et al., 1959).

In conclusion, bradykinin, in doses ranging from picograms to micrograms, or capsaicin stimulates receptors in the gallbladder of cats. Stimulation of these receptors reflexly activates the cardiovascular system through a spinalafferent pathway. Cardiovascular activation includes increases in blood pressure, systemic vascular resistance, heart rate, and myocardial contractility. Application of similar amounts of bradykinin or capsaicin to the serosal surface of the liver has no effect on the cardiovascular system. We have provided evidence that supports the rationale that, in inflammatory conditions, the formation of bradykinin in the gallbladder may stimulate a reflex activation of the cardiovascular system in several species including man.

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Amundsen E, Nustad K (1965) Kinin forming and destroying activities of cell hemogranates. J Physiol (Lond) 179: 479-488
Coleridge HM, Coleridge JCG, Kidd C (1964) Role of the pulmonary arterial baroreceptors in the effects produced by capsaicin in the dog. J Physiol (Lond) 170: 272-285
Frankish NH, Zeitlin IJ (1980) The effect of diet on tissue levels of kinin forming enzyme in blood free rat gastrointestinal tract. J Physiol (Lond) 298: 361-370

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