Hypotension and Release of Kinin-Forming Enzyme into Ascitic Fluid Exudate during Experimental Pancreatitis in Dogs

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HYPOTENSION IS an infrequent but severe complication of acute pancreatitis. A question remains as to whether the hypotension is a systemic manifestation resulting from the entry of pancreatic enzymes or toxins of pancreatic exudate into the circulation or whether the hypotension is the result of loss of plasma into the local inflammatory exudate in and around the pancreas. Clinical and experimental evidence has suggested that hypocalcemia,[7] oligemia[8] and circulating serum trypsin[1,3,4,12,14,16–20,23,26] might be responsible for the vascular collapse. Much of the literature[10,24,27,30] has dealt with the hypothesis that circulating trypsin is toxic because it activates the plasma kallikreinogen-kininogen system.

Previous studies from this laboratory[32] have suggested that although trypsin may be one of the hypotensive factors, other proteins in pancreatic juice may have hypotensive effects. Ofstad, *et al.*[13,21] reported that pancreatic exudate releases histamine from mast cells, and that the histamine may be a factor in the causation of the hypotension.

The purpose of the current investigation has been to compare the hypotensive effects of intravenously administered pancreatic juice with that of the exudative fluid formed during bile-induced pancreatitis in the dog; the hypothesis being that the leakage of pancreatic juice into the peritoneal cavity results in a greater release of kinin-forming enzyme than is present in the pancreatic juice itself.

In order to test this concept the kinin-forming activity of pancreatic juice was compared with that of the exudative fluid which accumulates during acute pancreatitis. Previous studies[32] have indicated that the escape of pancreatic juice into the circulation could not explain the profound hypotension observed during severe acute pancreatitis.

Material and Method

Normal pancreatic juice was collected from dogs through a glass cannula in the pancreatic duct inserted through a chronically implanted Thomas cannula in the duodenum. Subsequently, this juice was injected intravenously into other dogs according to the methods previously described.[6]

In other experiments, pancreatitis was induced by the injection of autologous bile (0.5 ml./Kg.) into the main pancreatic duct. The exudate was collected from the peritoneal cavity 3 hours after the induction of pancreatitis, and was injected intravenously into a second dog.

All infusions were made with the recipient dog under light sodium pentobarbital anesthesia. The mean arterial pressure was monitored from the femoral artery via a polyethylene catheter connected to a Sanborn pressure

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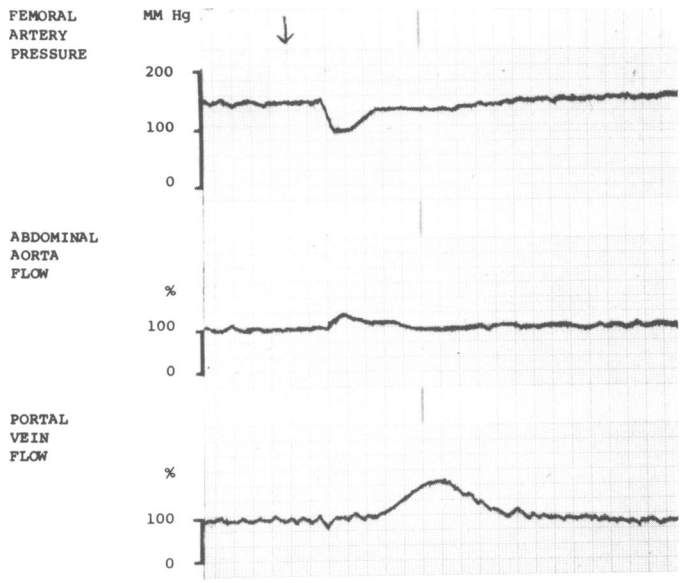

FIG. 1. Typical recording of blood pressure and blood flow after homoinfusion of normal pancreatic juice. Each major division represents 2 seconds.

transducer and recording system. Flow in the portal vein and in the abdominal aorta (above the renal arteries) was measured by a Biotronex Laboratory Model BL-610 pulsed-logic flowmeter.

Bradykinin in ascitic fluid and in normal pancreatic juice was measured according to the method of Talomo.[29] Ascitic fluid was withdrawn in disposable plastic syringes containing 3.6 M hexadimethrine and 9 mg. disodium ethylene-diamine-tetracetic acid E.D.T.A. Na_2) in 0.1 ml. of 0.15 M saline for each 9 ml. of samples. Pancreatic juice was collected directly into a plastic tube containing these materials. Samples were centrifuged immediately at 1000 g for 5 minutes at 4° C in the plastic tube. Aliquots of 2 ml. of supernatant were transferred with siliconized glass pipettes into plastic tubes. Protein was precipitated with 0.1 ml. of 20% trichloracetic acid. The tubes were then centrifuged at 1800 g for 5 minutes at 4° C. The supernate was decanted into a plastic tube. The precipitate was suspended in 1 ml. 0.9% trichloracetic acid and centrifugated again at 1800 g for 5 minutes. The supernates were combined and pH adjusted by the drop wise addition of 0.1 N NaOH to 3.5 to 4.0. The combined supernates were applied to a 4 × 0.5 cm column of Amberlite IRC-50 resin prepared in 0.1 N acetic acid. The sample was dried completely under reduced pressure, and subsequently determined for bradykinin by radioimmunoassay[29] and bioassay.

Kinin forming enzymes in normal pancreatic juice and ascitic fluid were measured using a substrate prepared according to the method of Jacobsen.[15] Bradykinin release by kinin-forming enzymes was measured largely by bioassay on rat uterus muscle. The uterine horn of a virgin female rat, weighing between 150–175 Gm. was employed in the muscle bath. The isotonic contraction of the muscle was recorded with a smoked drum kymograph. Synthetic bradykinin (BRS 640) supplied by Sandoz, Basel, Switzerland, was used as a standard. The Tyrode's solution contained 1 mg./ml. atropine sulfate and 1 mg./ml. of 1-methyl-d-lysergic acid butanolamide (UML-491) which is an anti-serotonin agent (Sandoz Pharmaceutical).

Enzyme analyses of pancreatic juice and ascitic fluid included amylase (Somogyi units),[28] lipase (Cherry-Crandall units)[5] and trypsin and trypsinogen (hydrohysis of benzol-DL-arginine p-nitroanilide (BAPA substrate).[9]

Specific Groups and Results

Group I: Homoinfusion of Normal Pancreatic Juice. Twelve homoinfusion experiments were performed on 12 recipient dogs weighing approximately 20 Kg. each. Pancreatic juice was infused intravenously in an amount totaling 50 ml. in each experiment. Each injection required approximately 10 seconds. The type of recordings obtained, which is typical of this series of experiments, is shown in Figure I. The mean arterial pressure and blood flow in the abdominal aorta and portal vein before, during, and after the infusion of pancreatic juice are shown in Figure 2. After each injection, a drop in mean arterial pressure, ranging from 14% to 50% of the preinfusion level, (mean 43.1% ± 4.1% S.E.) occurred after a mean latent period of 27.9 seconds (±2.5 S.E.). The blood pressure returned rapidly, within 20–200 seconds, to its control preinfusion levels.

After each injection, the blood flow in the aorta increased; the increment ranging 7–46% of the preinfusion flow with a mean increment of 33.4% (±3.4 S.E.). This increment in flow occurred after a mean latent period of 27.3 seconds (±2.1 S.E.). Within 50 to 100 seconds, the blood flow in the aorta was back to its control level.

Flow in the portal vein also increased, the increment ranging 14% to 50% of the control flow with a mean of 20.5% (±5.2 S.E.). This increased flow began after a mean latent period of 75.5 seconds (±7.0 S.E.). Within 80 to 120 seconds the portal flow returned to its preinfusion levels.

Group II: Infusion of Ascitic Fluid. Eight infusion experiments were performed on eight recipient dogs. The enzymes and bradykinin levels were measured in the infused fluid. Ascitic fluid, collected three hours after induction of pancreatitis, was infused intravenously in an amount of 50 ml. in each experiment as in the previous experiments.

The mean arterial blood pressure and the flow in the aorta and portal vein are shown in Figure 3. A drop of

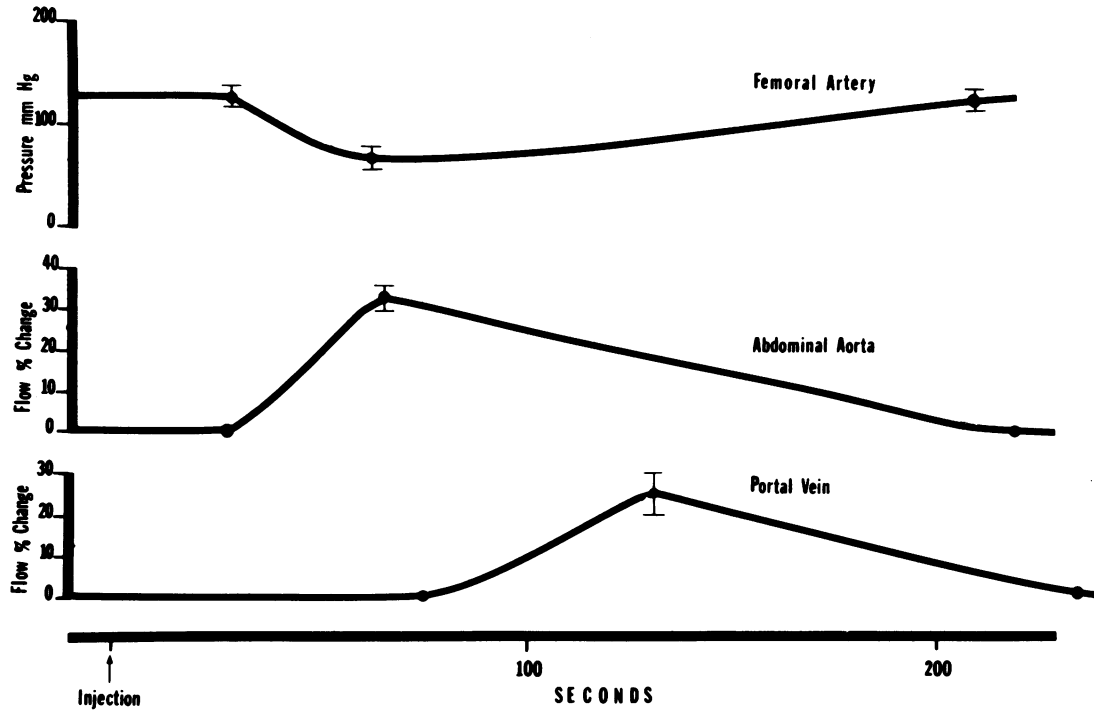

FIG. 2. Mean change (and standard error of the mean) of blood pressure and blood flow after homoinfusion of normal pancreatic juice.

mean arterial pressure occurred after injection, ranging from 4% to 52% of the control values, with the mean drop being 28.7% (±4.9% S.E.) after a mean latent period of 28.5 (±3.0 S.E.) seconds. The mean flow in the aorta and portal vein increased 28.3% (±6.2% S.E.) and 44.7% (±8.9% S.E.) respectively.

No bradykinin nor active trypsin could be found in normal pancreatic juice. Kinin-forming enzymes, however, were found in normal pancreatic juice, ranging from 1.3 to 43 ng/ml., with a mean level of 13.7 ng/ml. (±4.8 S.E.) (Table 1).

The volume of ascitic fluid collected three hours after

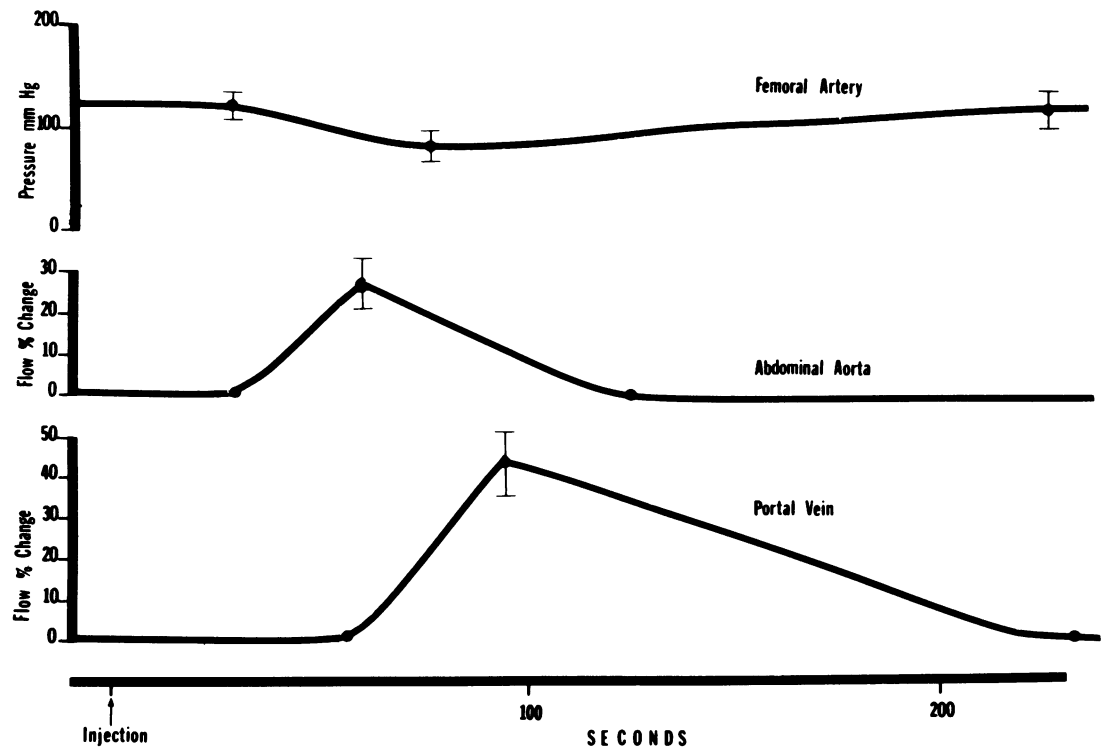

FIG. 3. Mean (and standard error of mean) changes of blood pressure and blood flow after infusion of ascitic fluid collected from acute pancreatitis.

TABLE 1. *Kinin-forming Enzymes in Pancreatic Juice*

Experiment Number	Kinin-forming Enzymes (ng/ml.)
1	1.3
2	25
3	2.9
4	2.5
5	5.5
6	8.6
7	21
8	43
Mean	13.7 (± 4.8 S.E.)

the induction of pancreatitis ranged between 350–700 ml., with a mean of 475 ml. (±36.6 S.E.) (Table 2). Amylase, lipase, and trypsinogen were present in high concentrations, but trypsin was detectable in only three instances. Bradykinin levels ranged between 0.3–2.4 ng/ml. with a mean of 0.99 (±0.4 ng/ml. S.E.). Since 50 ml. of ascitic fluid was infused into each dog it could be calculated that the amount of bradykinin infused (mean) was 49.5 ng. The level of kinin-forming enzyme ranged between 6.5–59 ng/ml., with a mean of 31.4 (±5.4 ng/ml. S.E.). The total (mean) amount of kinin-forming enzyme infused into each dog consequently was equivalent to 1570 ng of bradykinin.

Discussion

As mentioned earlier, much of the literature has dealt with speculation as to whether circulating trypsin is a toxic factor in acute pancreatitis, resulting in liberation of vasoactive substances through the plasma kallikreinogen-kininogen system. Previous studies in this laboratory[6,32] demonstrated that the recipient dogs could tolerate large amounts of trypsin or normal pancreatic juice given intravenously, repetitively, over periods as long as 2 to 3 months. As a result of these investigations, it was suggested that the hypotension of acute pancreatitis is probably not primarily the result of the absorption of normal pancreatic juice or of trypsin.

The present study has confirmed that the intravenous infusion of normal pancreatic juice in healthy dogs causes only a transient hypotension and in addition has shown that it causes an increased flow in the abdominal aorta and portal vein. Neither trypsin nor bradykinin was detected in normal pancreatic juice, but a fairly large amount of kinin-forming enzyme was detected. This kinin-forming enzyme is capable of releasing bradykinin and causing a temporary hypotension. It is known that the glandular kallikrein as contained in various animal tissues and fluids. Previous investigators[31] have demonstrated that the highest concentration of kallikrein could be detected in the pancreas of most animals except the rat and mouse.[31] The kinin-forming enzyme, which was detected in the current experiments, may be a glandular kallikrein secreted in pancreatic juice. Pancreatic damage, resulting from acute pancreatitis, apparently results in a release of glandular kallikrein from pancreatic tissue, which is known normally to contain 10–100 kallikrein units per gram of pancreas.[31]

The current study has demonstrated that the infusion of ascitic fluid exudate, collected during pancreatitis, causes a temporary hypotension and an increased flow in the aorta and portal vein which are similar, although less marked in the aorta, than that following the infusion of normal pancreatic juice. The hypotension effects of ascitic fluid, however, are probably important because of the relatively large volume of fluid which accumulated in the peritoneal cavity. The pancreatic enzyme levels in ascitic fluid were very high, but active trypsin was detected in less than half of the experiments. The hypotensive effect of ascitic fluid did not show any relationship to its trypsin content. Although only a small amount of bradykinin was found in ascitic fluid, a large amount of kinin-forming enzyme could be detected. The

TABLE 2. *Enzymes and Bradykinin in Ascitic Fluid*

Experiment Number	Ascitic Fluid ml.	Amylase Units/%	Lipase Units/ml.	Trypsin Units/ml.	Trypsinogen Units/ml.	Kinin-Forming Enzymes/ng/ml.	Bradykinin ng/ml.
1	450	62,000	19.9	4.8	59	18.9	0.7
2	700	365,000	12.3	1.5	27.7	10.0	0.3
3	500	75,000	5.8	0	16.0	35	0.7
4	550	750,000	9.4	0	11.0	75	0.7
5	400	120,000	7.2	0	26.0	20	2.4
6	450	70,000	5.6	0	18.0	43	1.8
7	350	320,000	10.4	0	14.0	20	1.0
8	400	70,000	9.3	3.5	6.5	30	0.3
Mean	475	229,000	9.48	1.22	22.27	31.46	0.99
±S.E.	±36.6	±80.26	±1.89	+0.65	±5.44	±6.76	±0.4

concentration of kinin-forming enzyme in the ascitic fluid was usually greater than that found in the pancreatic juice. This would imply that pancreatic exudate, escaping into the peritoneal cavity during acute pancreatitis, causes or is associated with the release of additional kinin-forming enzyme. Similarly Ofstad, *et al.*[13,21] demonstrated that pancreatic exudate, collected during acute pancreatitis, causes a temporary hypotension, when given intravenously in healthy dogs. This exudate was capable of releasing histamine from mast cells, and they postulated that the histamine might be the cause of the hypotension. Popieraitis, *et al.*[22] demonstrated that bradykinin, released during the course of experimental acute pancreatitis, is largely produced within the pancreas itself, rather than in any of the other glands of the body. From the current findings, however, it would appear that the escape of pancreatic juice into the peritoneal cavity contributes to only part of the kinin-forming enzyme activity found in the ascitic fluid. The kinin-forming activity of the ascitic fluid was greater than that detected in pancreatic juice. It therefore suggests that the pancreatic exudate causes a release of additional bradykinin and kinin-forming enzymes from other tissues within the peritoneal cavity or the injured pancreas releases more kinin-forming enzyme into the exudate than it does into normal pancreatic juice.

Peritoneal dialysis during acute pancreatitis has been reported to result in improved survival rate under clinical[2,11] and experimental conditions.[25] The current study suggests that the local accumulation of ascitic fluid, during pancreatitis, may play a contributing role to the hypotension of acute pancreatitis.

Summary

The hypotensive effects and kinin-forming enzyme content of pancreatic juice were compared with that of ascitic fluid exudate obtained from bile-induced pancreatitis in dogs. It was found that the hypotensive effects of pure pancreatic juice, collected from Thomas fistulae dogs, was slightly greater than that of an equal amount of ascitic exudate. Ascitic fluid, however, might contribute significantly to hypotension during acute pancreatitis since a large amount of fluid was observed to accumulate within the peritoneal cavity. This effect might be additive to the hypotension caused by the leakage of pancreatic juice directly into the circulation. The kinin-forming activity of the ascitic exudate was usually greater than that of pancreatic juice. This implies that the injured pancreas may release an additional amount of kinin-forming enzyme over and above that found in normal pancreatic juice. Another possibility is that the exudate causes a release of additional enzymes after its escape into the peritoneal cavity.

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