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Hemodynamic Characterization of a Canine Pancreatic Shock Factor[1] (41268)

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Abstract. A pancreatic shock factor (PSF) obtained by collagenase digestion was hemodynamically characterized in 37 mongrel dogs by measuring femoral artery pressure (FAP), portal venous pressure (PoVP), central venous pressure (CVP), pulmonary artery pressure (PAP), left atrial pressure (LAP), ascending aortic flow (cardiac output, CO), and portal venous flow (PoVF). Resistance was calculated for each vascular bed. Injections of PSF were made into the FAP, CVP, PAP, and PoVP catheters and compared. Other organs (muscle, lung, submandibular gland, liver, kidney, stomach, duodenum, and ileum) were processed similarly to the pancreas and the extracts hemodynamically tested. Also tested were known hypotensive agents: bradykinin, trypsin, and endotoxin. Small doses of PSF (0.002–0.004 ml/kg), injected at any of the sites, significantly decreased total systemic resistance, significantly increased portal venous resistance, and had no effect on pulmonary resistance. Cardiac depression was not seen as CO increased. Tachyphylaxis was not observed. The submandibular gland was the only other organ tested which possessed this vascular activity. Bradykinin produced a vascular response which mimicked the PSF or submandibular gland extract vascular response and tachyphylaxis was not observed. Trypsin and endotoxin did not mimic PSF as both depressed CO. The data suggest that kallikrein, present in both pancreas and submandibular gland, activates the kinin system to vasodilate the peripheral vascular bed with secondary effects of increasing CO and decreasing PoVF.

Profound hypotension quickly occurs when mixed pancreatic cell autotransplants, prepared by collagenase digestion, are placed into the portal vein. This phenomenon has occurred in all of our canine and human transplants (1). Our human autografts were not successful because shock developed before half of the cells could be infused. In dogs, we found that shock resulted from the injection of either the copiously washed cells or the supernatant from the collagenase digestion. Associated with the profound systemic hypotension was a marked increase in portal pressure sometimes associated with intestinal cyanosis. These vascular pressures mimicked those observed in endotoxic shock, however the cardiac depression of endotoxic shock was absent.

This report summarizes the results of canine studies. We sought to characterize the vascular response to a standardized pancreatic transplant supernatant by measuring regional blood flow changes; to determine a dose–response curve and thermal stability; to determine if the predigestion components (collagenase or minced pancreas alone) could account for the vascular response; and to detect if the pancreas was the only tissue containing a hypotensive agent.

Materials and Methods. Abbreviations for the hemodynamic parameters used in this test (all are expressed as mean values):

CO	cardiac output
CVP	central venous pressure
FAP	femoral artery pressure
LAP	left atrial pressure
PAP	pulmonary artery pressure
PAR	pulmonary artery resistance
PoVF	portal venous flow
PoVP	portal venous pressure
PoVR	portal venous resistance

[1] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. In conducting the research described in this report, the investigators adhered to the Guide for Laboratory Animal Facilities and Care, as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care, of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

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TPR total pulmonary resistance
TSR total systemic resistance

Canine monitoring model and preparation of pancreatic shock factor. Thirty-seven mongrel dogs of either sex, weighing 10–25 kg, received nothing by mouth for 24 hr. Each dog was placed dorsally on a heating blanket and anesthetized under light endotracheal halothane anesthesia. The vascular access lines were introduced (Fig. 1). A Swan–Ganz catheter was inserted through an external jugular vein and guided into the pulmonary artery by digital control while the chest was opened for placement of a left atrial pressure (LAP) cannula. Through the Swan–Ganz and LAP cannulas, central venous pressure (CVP), pulmonary artery pressure (PAP), and LAP were continuously monitored via pressure transducers (Statham Instruments, Inc., Medical Division, Oxnard Calif.) with a Honeywell Visicorder and video screen (Honeywell, Test Instruments Division, Denver, Colo.). Another catheter was placed in the femoral artery to monitor arterial blood pressure. Arterial blood gases were monitored every 30 min and the pH

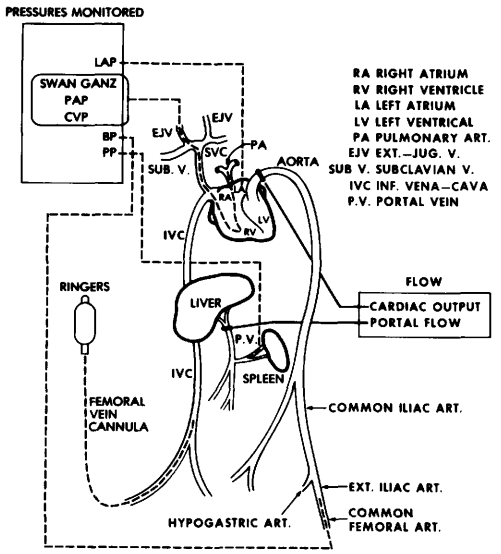

FIG. 1. The canine monitoring model used for pressure and flow measurements in order to calculate resistance in the portal, pulmonary, and systemic vascular beds.

was kept at 7.4 ± 0.1 with respirator adjustment or sodium bicarbonate.

After the lines were placed and the animal was hemodynamically stable, the abdomen was opened and the uncinate process, body, and tail of the pancreas were removed and immediately placed in iced Hanks' balanced salt solution (HBSS, Gibco, Grand Island, N.Y.). A splenectomy was not performed to avoid altering the normal canine splanchnic vascular response of splenic contraction with hypotension and catecholamine release (2). The volume of wet pancreatic tissue was determined. The pancreas was then minced with scissors on ice, finely chopped to 1-mm fragments with a mechanical tissue chopper in iced HBSS, and washed three times with 500 ml of iced HBSS per wash. After the final wash, HBSS was added to the tissue fragments so that the final volume represented one and a half times the original wet pancreatic tissue volume. This served to standardize the volume and enzyme activity of the supernatant used for intravascular injections, and is subsequently termed pancreatic shock factor (PSF). Pancreatic fragments were then digested by using 1440 units of collagenase (Worthington Type IV, Worthington Biochemical Corp., Freehold, N.J.) per milliliter of wet pancreatic volume in a shaking water bath at 37° for 20 min. The mixture was immersed in ice for 2–3 min. The supernatant from the digestion mixture was decanted and centrifuged at 800*g* at 0° for 5 min, decanted, and then stored in an iced Erlenmeyer flask.

While the pancreas was being processed, a portal venous catheter was placed through a splenic vein branch for administration of the PSF and monitoring portal pressure. In addition, using Carolina flow probes and meters (Carolina Medical Electronics, Inc., King, N.C.), we monitored mean blood flow in the ascending aortic arch (cardiac output) and the main portal vein.

Experimental design. To determine the typical vascular response and the site of action, a total of 10 animals was studied. After all pressures and flows were stabilized, each animal received a sequence

of four 2.0-cc injections of PSF. One injection was placed into each of the following sites in the order listed: portal vein, right atrium, left atrium, and femoral artery. All parameters were monitored continuously following each injection. Pressures and flows were allowed to return to baseline values before additional injections of PSF. Systemic, total pulmonary, and portal venous resistance (dyn-cm-5) were calculated according to formulas as outlined by Yang *et al.* (3).

$$\text{TSR} = (\text{FAPm} - \text{CVPm})/\text{CO},$$
$$\text{TPR} = \text{PAPm}/\text{CO},$$
$$\text{PoVR} = (\text{PoVPm} - \text{CVPm})/\text{PoVF}.$$

Statistical significance was determined using Student's t test comparing baseline resistance values to their maximal deviation (4).

To calculate a dose–response curve, five additional dogs prepared as in Fig. 1 received 23 dilutions of PSF into the portal vein (0.001–0.01 cc/kg brought to a 2-cc volume with HBSS). Again flows and pressures were allowed to return to baseline values before additional injections.

A dose–response curve was obtained for bradykinin (Sigma Chemical Co., St. Louis, Mo.) in a similar manner using five additional dogs. Nineteen increasing doses of bradykinin (12.8–1280 ng/kg) were infused into the portal vein.

To determine if the original constituents of the pancreatic supernatant before digestion possessed vascular activity, the following components were tested via the portal vein using five additional dogs prepared as in Fig. 1: two cubic centimeters of either iced HBSS alone, minced pancreas alone, or a collagenase solution equal to the digestion concentration.

From the five dogs subsequent to the latter experiment, eight organs or tissues were removed and processed in the same manner as the pancreas to determine if these tissues possessed hypotensive activity. Supernatants were obtained from muscle, lung, submandibular gland, liver, kidney, stomach antrum, duodenum, and ileum. The eight supernatants from these tissues plus a pancreatic supernatant from the five dogs were tested for *in vivo* vascular effects by injection into the portal vein of two additional dogs prepared as in Fig. 1.

Solutions which might produce vascular responses which mimic the response to PSF were tested through the portal vein in an additional five canine monitoring models: bradykinin (pH 7.0, 256 ng/kg (5); trypsin (Type 1) (pH 6.8), 800 units/kg; and *Escherichia coli* endotoxin (serotype 055: B5) (pH 6.6), 1 mg/kg (all from Sigma Chemical Co.). These agents dissolved in normal saline were administered at room temperature.

The vascular response to PSF was further investigated in five additional canine monitoring models. Two-milliliter aliquots of PSF were tested after either freezing to 0° or heating for 30 min (40, 60, 80, and 100°). A paired control injection of unfrozen or unheated PSF was used before each frozen or heated PSF injection.

Results. *Hemodynamic responses.* Separate injections of PSF directly into either the liver (main portal vein), lungs (CVP line), systemic vascular system (LAP catheter), or regional systemic vascular bed (femoral artery) resulted in significant changes from baseline values of vascular resistance to a maximal deviation. Figure 2 depicts the resistance changes using the portal vein injection site. The other injection sites were associated with similar vascular resistance changes, so are not depicted. The P values for these resistance changes are listed in Table I. A significant decrease in TSR followed by a significant increase in PoVR was seen after the PSF was injected at each site. The level of significance comparing baseline to maximal PoVR deviation steadily decreased as the injection was placed farther from the portal system and was of only borderline significance when the femoral artery was used. A statistically significant change was not observed in PAR or TPR regardless of the injection site.

The sequence for the hemodynamic changes was similar regardless of the injection site; i.e., a fall of FAP, an increase in CO, a decrease in PoVF, an increase in

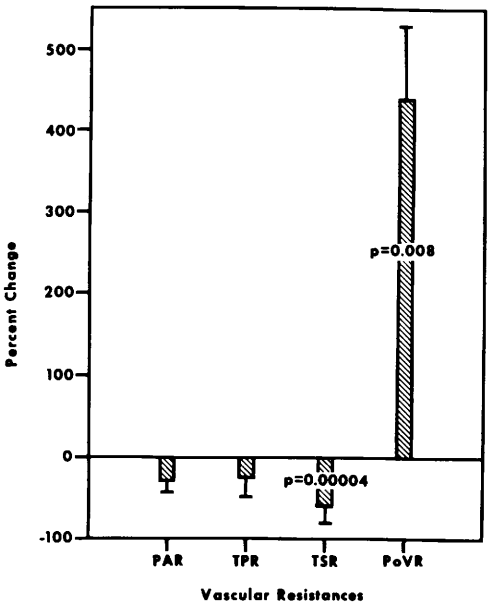

FIG. 2. The percentage change of vascular resistance (dyne cm^{-5}) from baseline values to maximum change after injection of PSF into the portal vein. The *P* values were calculated using the actual resistances and are depicted only if statistical significance of <0.05 was achieved.

LAP and PAP, followed much later by an increase in PoVP (Table II). The first reaction to PSF, the fall in FAP, occurred significantly much sooner if the injection site was the CVP, LAP, or FAP cannula than if the site was the portal vein ($P < 0.05$). This relationship also held true when the times for onset of reaction for the decreased CO were observed for each injection site. The dynamic time relationships between changes of FAP, CO, and PoVP are shown in Fig. 3.

A dose–response curve was constructed after portal vein injection of PSF (Fig. 4). As little as 0.002 ml/kg of PSF produced a significant change from baseline in CO and 0.004 ml/kg produced a significant change in FAP and TSR. Tachyphylaxis was not observed.

The dose response to bradykinin after portal vein injection also did not show tachyphylaxis. A statistically significant ($P < 0.05$) change in baseline values occurred (Fig. 5) in FAP (64 ng/kg), CO (512 ng/kg), and PoVP (896 ng/kg) after injection of bradykinin.

Anatomical and chemical origin of PSF. Injection of iced HBSS, undigested minced pancreas, collagenase solution alone, or a standardized supernatant from all of the nonpancreatic tissues (except submandibular gland and stomach) did not result in vascular pressure or flow changes. The response to the submandibular gland-standardized supernatant exactly mimicked the PSF response. There was a mild fall in cardiac output (mean 16.5 ± 5.0%) after injection of supernatant from stomach antrum.

Thermal stability of PSF. Freezing produced no effect on the vascular response to PSF. Heating to 80° for 30 min partially inactivated PSF, but 100° was necessary to inactivate PSF totally.

Potential vasoactive candidates for PSF. When bradykinin was administered into the portal vein of five dogs, a sequence of vascular events occurred as after administration of PSF (Fig. 3). The administration of trypsin or endotoxin in five animals each produced a decrease in FAP, an increase in PoVP, but a decrease in CO (Fig. 6).

Discussion. The vascular response to PSF begins 10 sec (LAP catheter injection) to 17 sec (portal vein injection) after a bolus infusion. The initial change is a profound systemic hypotension followed in 2 to 3 sec by an increase in CO. This increase in CO is accompanied in 8 to 10 sec by a decrease in PoVF. The decrease in TSR and increase in PoVR are statistically significant when compared to baseline values.

The peripheral vascular bed is the main site of PSF activity because the FAP was the first parameter to change regardless of the injection site. The shortest time of onset for systemic hypotension was after injection of PSF just proximal to (CVP line or LAP cannula) or directly into (femoral artery) the peripheral vascular system.

The decrease in PoVF following a fall in TSR is explained by a physiologic reflex. As the TSR falls, aortic and presumably hepatic artery flow increase but, to maintain the original total hepatic blood flow, portal vein flow must decrease (6). The portal venous constriction associated with decreased PoVF redistributes portal blood volume into the systemic veins increasing

TABLE I. *P* VALUES FOR RESISTANCE CHANGES AT VARIOUS INJECTION SITES

Resistance	Injection site			
	PV	CVP	LAP	FA
PAR	ns[a]	ns	ns	ns
TPR	ns	ns	ns	ns
TSR	0.0004	0.00006	0.0007	0.003
PoVR	0.008	0.015	0.014	0.048

[a] Nonsignificant.

venous return (7). The time sequence of a change in FAP and CO followed by a PoVF change would confirm that the new portal venous hemodynamics are secondary to the decreased TSR. Regardless of the injection site, PoVF decreases 5 to 6 sec after the drop in FAP. The increase in PoVR was not as marked when PSF was introduced farther away from the portal system until borderline significance ($P < 0.05$) was achieved when the femoral artery was used. This latter maneuver prolongs entry of PSF into the entire peripheral vascular bed and allows the greatest time for intravascular degradation.

The site of action for this pancreatic hypotensive agent is probably the peripheral vasculature with a compensation for the systemic vasodilation by increasing cardiac output through baroreceptors. Portal blood volume is probably added to the systemic system by portal venoconstriction increasing venous return and helping to increase FAP.

The increase in CO after PSF indicates an absence of cardiac depression, as was observed after infusion of endotoxin or trypsin. This compensatory rise in CO following peripheral vasodilation indicates little similarity to the myocardial depressant factor (MDF) described by Lefer that arises late in shock and is thought to arise in the pancreas (8).

The increase in PoVR could be secondary to particulate matter in the PSF. A cellular suspension would definitely act as emboli in the portal venules; however, undigested minced pancreas injected into the portal vein resulted in no change in PoVR. The PSF following digestion and centrifugation is still accompanied by an increase in PoVR following systemic vasodilation.

Each component of the collagenase digestion mixture was investigated for vascular effects. Iced HBSS, undigested minced pancreas, or a collagenase solution alone (concentration equal to the digestion mixture) had no effect on the vascular system. Evidently, pancreatic tissue and collagenase combine to produce the vasoactive factor. Small amounts of collagenase added to pancreatic homogenate activates tryptic activity (9). Trypsin is not a candidate for PSF because, in our past experience, copiously washed pancreatic cells retain PSF activity but have low tryptic activity. Also the depressed CO following infusion of trypsin did not mimic PSF. The vascular response to PSF is not from individual components of collagenase digestion used to prepare pancreatic mixed cell suspensions, but rather a result of their interaction.

PSF retains its activity after freezing or heating. This suggests the presence of a nonenzymatic compound which activates a

TABLE II. TIME FOR ONSET OF REACTION

	Reaction	Injection site			
		PV	CVP	LAP	FA
FAP	↓	17.5 ± 1.5[a]	11.7 ± 1.05*	10.7 ± 0.6**	11.5 ± 0.8*
CO	↑	19.5 ± 2	14.1 ± 1.3*	12.6 ± 0.8**	14.7 ± 1.3*
PoVF	↓	25.5 ± 4.1	26.5 ± 5.3	19.3 ± 3.6	21.1 ± 4.1
PAP	↑	25 ± 2.7	21.8 ± 2.7	21.7 ± 3.2	31.4 ± 7.1
LAP	↑	29.3 ± 4.8	26.5 ± 4.7	27.5 ± 3.4	35 ± 7.6
PoVP	↑	36.5 ± 6.3	37 ± 7	41.3 ± 4.9	52.8 ± 8.2

[a] sec, mean ± SEM.
* Significant when compared to PV injection site, $P < 0.05$.
** Significant when compared to PV injection site, $P < 0.01$.

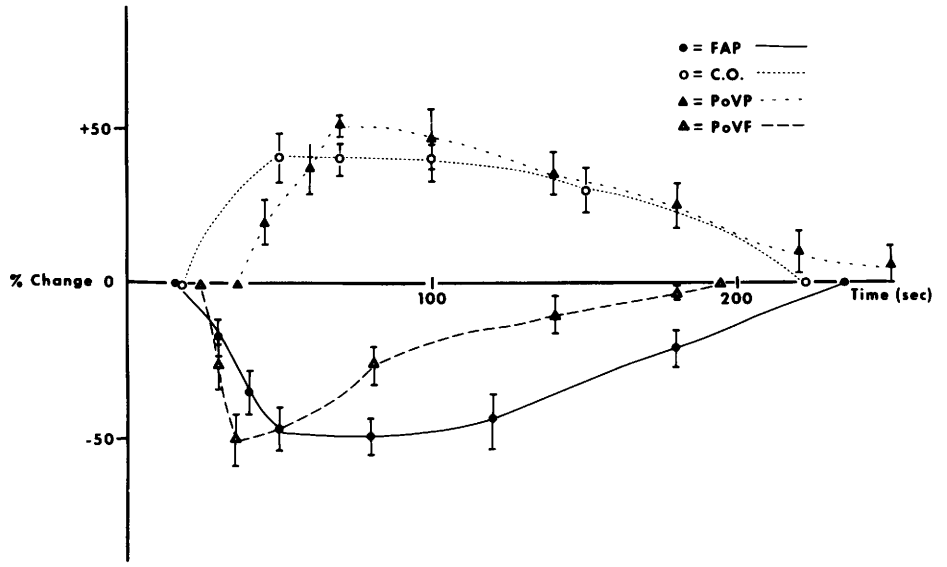

FIG. 3. A typical response to portal vein injection of standardized pancreatic shock factor illustrating the initial change in FAP and CO quickly followed by a decrease in PoVF and lastly PoVP.

vasodilating precursor. Endotoxin is heat stable but the *in vivo* depression of CO by endotoxin or trypsin does not mimic the increasing CO following PSF. The vascular response to bradykinin does mimic the PSF reaction. Bradykinin increases CO either by directly increasing myocardial contraction or by reflex activation of the sym-

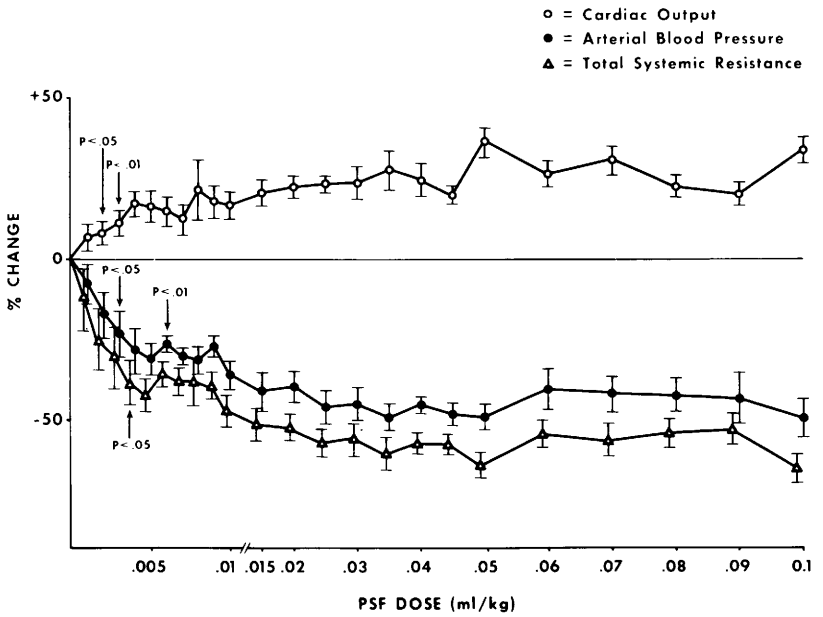

FIG. 4. A dose–response curve showing the decrease in arterial blood pressure (FAP), TSR, and CO with increasing doses of standardized PSF per kg in five dogs. Significant changes (indicated by arrows) from baseline occurred for CO at 0.002 ml/kg ($P < 0.05$) and 0.003 ml/kg ($P < 0.01$), FAP at 0.03 ml/kg ($P < 0.05$), and 0.05 ml/kg ($P < 0.01$), and TSR at 0.004 ml/kg ($P < 0.05$). Tachyphylaxis is not present.

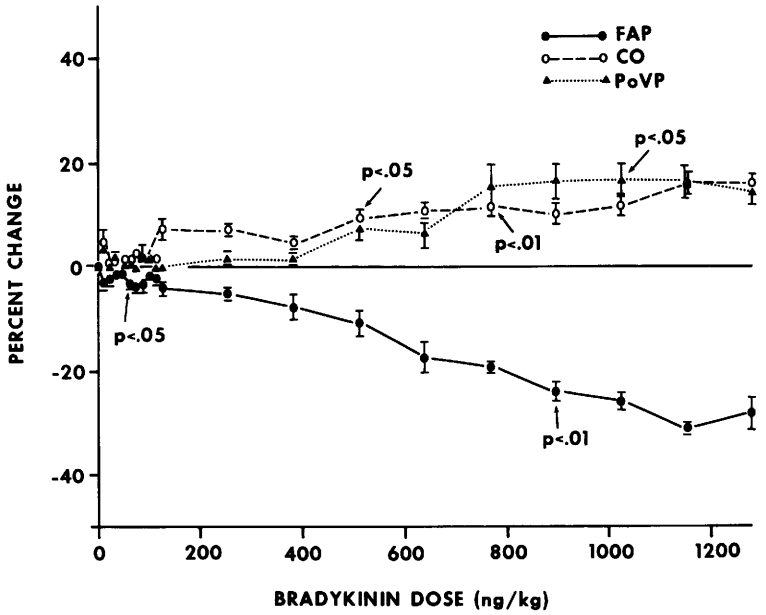

FIG. 5. A dose–response curve shows an absence of tachyphylaxis to increasing doses of bradykinin. Statistical significance (arrows) from baseline values was achieved with small bradykinin doses in femoral artery pressure (FAP, 64 ng/kg), cardiac ouput (CO, 512 ng/kg), and portal venous pressure (PoVP, 896 ng/kg). Significance was extended to the $P < 0.01$ level with higher doses of bradykinin in blood pressure (896 ng/kg) and cardiac output (768 ng/kg).

pathoadrenal system from hypotension (10). The pulmonary resistance was relatively unaffected which suggests either the main site of action for PSF was not in the lung and/or that the pulmonary vessels have a unique tissue-bound inactivating system. The lungs may destroy up to 90% of kinins during their first circulation through the pulmonary system (11).

Except for submandibular gland, the

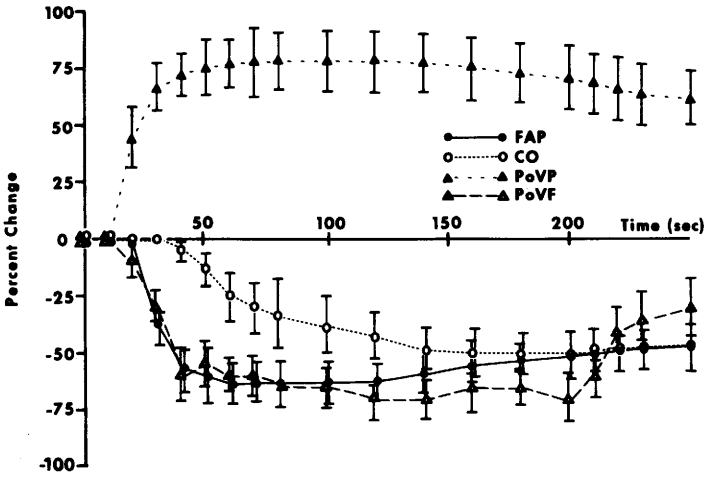

FIG. 6. Injection of trypsin decreases FAP and PoVF but also decreases CO. The latter is not typical of the response to PSF or bradykinin. A similar reaction to trypsin occurred when endotoxin was injected into the portal vein, but is not depicted.