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Severe intestinal ischemia can trigger cardiovascular collapse and sudden death via a parasympathetic mechanism

Alexander H. Penn and Geert W. Schmid-Schönbein

Department of Bioengineering, The Institute for Engineering in Medicine, University of California San Diego, La Jolla, CA, 92093-0412

Abstract

Hemorrhagic shock (HS) and splanchnic arterial occlusion (SAO) followed by reperfusion are associated with high mortality. However, rapid cardiovascular failure and death may also occur prior to reperfusion in HS and SAO. We show in a rat SAO model that upon gut ischemia, mean arterial blood pressure transiently elevates and then drops fatally in one of two time courses: (i) gradually over ~1 to 3 hours or (ii) rapidly (often by more than 80 mmHg) over a period of 1 to 6 minutes. We hypothesize that fast fatal pressure drops (FFPD) are due to failure of autonomic nervous system control. To test this, we treated rats with glucose (10%) in the small intestinal lumen and intramuscular xylazine to activate the parasympathetic nervous system, or with a muscarinic anti-cholinergic (glycopyrrolate) or by total subdiaphragmatic vagotomy (TSV) to attenuate parasympathetic nervous system activity. We also tested nafamostat mesilate (ANGD), a protease inhibitor efficacious in preventing blood pressure loss in SAO *with reperfusion*, in the intestinal lumen. 50% of animals receiving xylazine and glucose died by FFPD (vs. 33% with neither, NS).

TSV or glycopyrrolate treatment significantly reduced the incidence to 0% ($P < 0.008$), though slow fatal pressure drops (SFPDs) still occurred. ANG D did not prevent FFPDs, but delayed onset of SFPDs ($P < 0.013$). These results suggest that gut ischemia can cause sudden death via an autonomic nervous system mechanism and that SAO with glucose and xylazine may serve as a useful model for study of neurogenic shock or autonomic dysregulation associated with sudden death.

Keywords

autonomic nervous system; vagotomy; glycopyrrolate; splanchnic arterial occlusion; gut; neurogenic shock; sudden cardiac death; sudden infant death syndrome

Introduction

Hemorrhagic shock and gut ischemia are associated with high mortality. In rat models of shock, two hours of hypovolemia or splanchnic arterial occlusion (SAO) followed by return of shed blood or release of the occlusion usually cause a decline in blood pressure and death

Correspondence: Dr. Alexander H. Penn, Department of Bioengineering, The Institute for Engineering in Medicine, University of California, San Diego, La Jolla, CA 92093-0412 USA, Tel: 858 534-2714, Fax: 858 534-5722, apenn@bioeng.ucsd.edu.

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within a few hours after the onset of reperfusion (1, 2). However, the mechanisms of blood pressure reduction that lead to death are currently unknown.

We have obtained evidence in models of shock, including two hour SAO followed by reperfusion, hemorrhagic shock, and endotoxic shock, that central blood pressure reduction is associated with translocation of digestive enzymes from the lumen into the wall of the intestine and with formation of inflammatory mediators (1, 3–5). The enzymes and inflammatory mediators are transported via the portal venous circulation, the lymphatics, and the peritoneum into the central circulation and cause remote organ injury beyond the intestine (4, 6, 7). Inhibition of the pancreatic digestive enzymes by different digestive enzyme inhibitors, results in significantly improved blood pressure and reduced inflammation (1, 3–5).

However, in addition to death from a gradual reduction of blood pressure after gut ischemia and reperfusion due to hypovolemia or SAO, we observed that death may also occur *during* ischemia following a precipitous drop in blood pressure. We designate such pressure drops as fast fatal pressure drops (FFPDs). Though the gradual pressure drop and death that occurs *after* reperfusion has been linked to pancreatic digestive enzymes, there may be a mechanism involved in the FFPDs that is not directly linked to digestive enzymes.

The autonomic nervous system controls blood pressure by adjusting vascular tone. The sympathetic subsystem has the ability to facilitate moment-to-moment blood pressure adjustments, such as quickly raising pressure during rapid transition from supine to standing position. The parasympathetic system, in contrast, can lower systemic pressure and also buffers rapid fluctuations in blood pressure triggered by changes in the sympathetic nervous system (8). Gut ischemia is known to activate the sympathetic nervous system via spinal nerve afferents resulting in increased blood pressure (9).

Furthermore, the autonomic nervous system detects glucose absorption in intestinal epithelium and by the presence of glucose in the portal vein resulting in increased parasympathetic nervous system activity (10, 11). In pilot studies of SAO, glucose added into the lumen of the intestine increased incidence of mortality by FFPD prior to reperfusion. Consequently we hypothesize that the mechanism for FFPDs in SAO may involve the autonomic nervous system and is similar to that of “neurogenic shock”, a form of hypotension that results from loss of sympathetic control of vascular tone with a relative bradycardia and uncompensated parasympathetic activity, as in spinal injury (12).

Given that reperfusion is not always immediately possible, for example on the battlefield, in remote locations, in surgical conditions when occlusion is necessary (e.g. during aortic reconstructions), or when reperfusion would lead to further bleeding, we wished to determine the cause of rapid death during ischemia. The aims of this study were therefore to determine in a SAO model of intestinal ischemia the incidence of death by a fast fatal pressure drop, and to determine whether the autonomic nervous system or pancreatic digestive enzymes are involved in the blood pressure drops. To accomplish this, we analyzed in detail blood pressure histories during gut ischemia in conjunction with a variety of treatments including: glucose in the intestinal lumen and/or intramuscular xylazine to stimulate the parasympathetic nervous system; total subdiaphragmatic vagotomy or addition of glycopyrrolate, an anti-cholinergic, to interfere with the parasympathetic nervous system; and 6-amidino-2-naphthyl p-guanidinobenzoate dimethanesulfonate (ANGD, nafamostat mesilate, Torii Pharmaceutical, Chiba, Japan) to block pancreatic digestive enzymes in the lumen of the intestine.

Materials and Methods

To identify the treatments and conditions tested, animal group designations were selected to be modular in nature. For example, animals in the *Xyl Gly Glucose* group received xylazine, glycopyrrolate, and glucose prior to undergoing SAO.

Animals and Surgical Procedures

All animal protocols were reviewed and approved by the University of California San Diego Animal Subjects committee. Male Wistar rats, after a minimum of 5 days non-stressful existence in our facility, were given general anesthesia (sodium pentobarbital, 50 mg/kg, i.m.) and kept warm on a heated surgical table. Rats in the *Xyl* groups were also tranquilized with xylazine (4 mg/kg i.m.) 20 minutes prior to general anesthesia. One group of *Xyl* animals also received glycopyrrolate (0.5 mg/kg, i.m., *Xyl Gly Glucose* group) immediately after xylazine injection. Since reported cardiovascular effects of xylazine include initial hypertension followed by sustained hypotension and increased vagal tone-related bradycardia (13), we also included groups that did not receive xylazine (*Saline* and *Glucose* groups) to determine the effects of ischemia without parasympathetic nervous system stimulation by xylazine.

All animals were cannulated via the left femoral vein for supplemental anesthetics as needed by reflex test and via the left femoral artery for fluid collection and pressure measurement. Arterial blood was collected in 10 U/ml (final) of sodium heparin and centrifuged (1000g, 10 min) to obtain plasma, which was frozen (-20°C) for later measurement of plasma glucose levels and protease activity.

Animals in the *Total Subdiaphragmatic Vagotomy (Xyl TSV Glucose)* group also received after laparotomy a left subcostal incision (after ligation of the superior epigastric vessels to prevent bleeding). In these groups, we ligated the esophagus in two spots approximately 3 mm apart below the diaphragm and performed an esophagotomy between the ligatures, thus cutting both the anterior and posterior trunks of the vagus nerve positioned alongside the esophagus.

Shock Protocols

In all groups, we exteriorized the small intestine and cecum and sandwiched them between two pieces of gauze soaked in warm saline (37°C), placed alongside of the animal, and covered in plastic wrap to maintain body temperature and moisture. The superior mesenteric and celiac arteries, which serve to perfuse the small intestine and in part the liver, pancreas, spleen, and stomach, were isolated with umbilical tape. 6 to 10 ml (volumes were adjusted to provide fluid along the entire length of the small intestine with minimal stretching of the intestinal wall) of saline (37°C) (*Saline* groups), 100 mg/ml glucose in saline (*Glucose* groups), or the protease inhibitor ANG2 (2 mg/ml in 100 mg/ml glucose in saline; *Glucose ANG2* group) were injected into the intestinal lumen at two or three sites along the small intestine. Luminal injections were completed in less than 5 min. Animals in the *Xyl Gly Glucose* group received additional glycopyrrolate (0.5 mg/kg, i.v.) at this time.

Within seconds of completing the luminal injections, SAO was initiated (with the exception of the *Xyl Gly Glucose* group which was allowed to adjust to the i.v. glycopyrrolate for 15 minutes prior to occlusion). Occlusion was achieved by tying the lengths of umbilical tape isolating the superior mesenteric and celiac arteries around short pieces of polyethylene tubing placed adjacent to the arteries to occlude with minimal damage to the blood vessels. Due to its close proximity to the superior mesenteric artery, the main lymphatic vessel exiting the intestine was also isolated and occluded together with the superior mesenteric artery. Pilot studies (N=3) showed that the weight of the small intestine plus its luminal

content does not change from fluid loss or gain during a 2 hr ischemia, even if followed by reperfusion for 2 hrs, excluding the possibility that the central blood pressure reduction was due to fluid loss into the lumen of the intestine. No artery/arteriolar or venous/venular bleeders were detected in any of the animals in this study. An additional group (n = 3 animals) received xylazine and glucose treatments but no occlusion of the arteries (*Xyl/ Glucose Sham* group).

The wet gauze and plastic wrap were adjusted to cover the intestine and the abdominal opening, leaving the small intestine outside the body for the remainder of the experiment to allow direct observation of hemorrhage within the intestine and prevent possible leakage of mediators from the wall of the intestine into the peritoneal space. Each animal was then left undisturbed throughout the experiment. Death was declared when mean arterial blood pressure (MABP) reached less than 20 mmHg with pulse pressure below 1 mmHg.

Tissue Analysis

After 4 hours ischemia or at death, whichever came first, an aliquot of blood was collected into sodium heparin (10 U/ml, final) via the arterial catheter if blood pressure was over 40 mmHg (enough pressure to allow blood collection through the catheter) or via atrial cardiac puncture if the pressure was below 40 mmHg or if the animal was deceased. At 4 hours, surviving animals were euthanized (120 mg/kg sodium pentobarbital, i.v.).

Whole blood samples were centrifuged (1000g, 10 min) and the plasma stored (-20°C). High levels of hemolysis were observed in the blood obtained from cardiac puncture from two animals and these were excluded from protease analysis due to interference with the zymographic techniques. Organs were inspected for macroscopic signs of edema, tissue hemorrhage, and other lesions.

Plasma glucose levels were obtained with a commercial glucose meter (Contour Meter, Bayer Health Care, New York). Plasma protease activity levels were determined with a fluorescently labeled casein substrate (E6639 Enzchek Protease Assay Kit; Invitrogen, Carlsbad, CA) after 90 min reaction at 37°C in a 96-well plate fluorescence reader (Spectramax Gemini XS; Molecular Devices, Sunnyvale, CA).

Continuous arterial blood pressure histories were digitally recorded every 0.001 seconds (Maclab, ADInstruments, Colorado Springs, CO), from which heart rate, systolic, diastolic, and mean blood pressures for each pulse were computed and stored for statistical analysis (Microsoft Excel).

Arterial Blood Pressure Analysis

To examine early differences between groups we noted hemodynamic parameters immediately after femoral artery catheterization, immediately before arterial occlusion, at peak initial MABP (usually within the first 5 minutes of occlusion) and at 20, 40, and 60 minutes during occlusion.

To analyze the full pressure histories, we fit the MABP trace with a series of linear segments. This approach provides a close approximation to the measurements since most MABP traces are piecewise linear and had clearly identifiable transitions between segments with different slopes (Figures 1). In some animals, the blood pressure rapidly fluctuated around an average trend for varying lengths of time (e.g. Figure 1 **bottom**). In those cases, we placed the line segment along that average trend.

In order to compare pressure histories between experimental groups, we grouped line segments into different time/pressure *periods* based on common characteristics among the

pressure histories (Figure 1). For example, immediately after occlusion, blood pressure rises sharply (for less than a minute), followed usually by a slower rise (for several minutes). We designated this, collectively, the *Initial rise period*. After a few minutes of relatively stable MABP, blood pressure begins to drop in the *Initial drop period*. In animals treated with xylazine, the pressure on average drops more sharply at first then levels off as it approaches the pressure before occlusion (see Figure 1 **upper and middle left**). The end point of the initial drop was determined as the first time following its onset at which the trend of the MABP maintained a stable or positive slope (i.e. roughly greater than -0.5 mmHg/min for 4 or more min).

Following the *initial drop period*, the pressure histories vary from animal to animal as MABP goes through a series of stable pressures, gradual pressure drops, gradual pressure recoveries, and occasional sharp drops followed usually by a rapid pressure recovery of similar magnitude. Despite frequent pressure drops and recoveries in many animals, the overall pressure change in this period is usually small.

The *fatal pressure drop* represents the final blood pressure reduction bringing the MABP below 40 mmHg, a level at which death follows soon afterwards. All but 2 of the animals exhibited a fatal pressure drop. The majority of fatal pressure drops fit into either of two classes: a fast drop (Figure 1 **upper left and middle right**) or a slow drop (Figure 1 **upper right and middle left**) as defined by the length of time between the start of the fatal pressure drop and death (see Table I). The transition into the start of a FFPD was usually very clear and could be pinpointed to within a minute (and sometimes within a second). The transition into a SFPD was not always fully evident, as sometimes there was more than one transition from line segment to line segment that could be viewed as the onset. In those cases, we chose the transition involving the larger change in slope. These potential onset times were usually within 10 min of each other.

Though the last few line segments following the fatal pressure drop vary from animal to animal, with some accelerating towards no detectable blood pressure and others leveling off for a while or even briefly recovering, a MABP below 40 mmHg was rapidly followed by death regardless of the type of fatal pressure drop.

When more than one line segment in a pressure history was part of a given period, we calculated the period's total duration and pressure change as the sum of the segment durations and pressure changes. For example, in Figure 1 **top left**, while the *Initial drop* period is shown as a single line that is not superimposed over the blood pressure trace, it represents the sum of three sequential line segments that did superimpose over the trace. The period's overall slope was calculated as the total pressure change divided by the total duration.

Statistics

Results are presented as mean $\pm$ standard deviation. The average blood pressure histories were plotted using only the start and end points of the initial rise, initial drop, and fatal pressure drop, and the time of death. However, all statistical analysis was performed comparing the exact start and end times, start and end blood pressures, durations, slopes, and pressure change of each period.

For statistical comparisons between two groups (e.g. FFPD animals vs. SFPD animals) a Student's T-test was used with $P < 0.05$ considered significant. To compare mean values between multiple groups, we first performed ANOVA over all 7 SAO groups, then used a Bonferroni corrected Student's T-test for pair-wise comparisons. As each group was

compared to either one or two others (e.g. Xyl Saline ↔ Xyl Glucose ↔ NoXyl Saline ↔ NoXyl Glucose ↔ NoXyl Glucose ANG), $P < 0.025$ was considered significant.

Within the 4-hour observation period, 13 of the total of 77 animals subject to SAO clearly started a fatal pressure drop but did not complete it (i.e. they were still alive at 4 hours) (Figures 2 and 3 captions). For these animals, we estimated the time of death by extrapolating the pressure drop at the final recorded rate to a value below 20 mmHg MABP, since we observed animals always died within a few minutes of reaching 20 mmHg.

Two animals had no fatal pressure drops within the 4-hour observation period and were therefore excluded from fatal drop and time of death comparisons (Figures 2 and 3 captions). These animals did not differ within their groups by weight, time spent in vivarium, or details in the surgical technique.

Significant changes in FFPD incidence were determined by chi-square analysis ($P < 0.025$ considered significant as this was also a comparison between multiple groups).

Results

Effects of xylazine and glucose on hemodynamics and blood glucose levels

Both xylazine and glucose revealed effects suggesting parasympathetic nervous system activation. Animals receiving xylazine had a significantly lower mean arterial blood pressure (MABP; 79.1 ± 6.2 vs. 94.3 ± 11.3 mmHg), heart rate (246.2 ± 37.3 vs. 333.1 ± 35.9 bpm), and pulse pressure (40.5 ± 3.4 vs. 43.9 ± 6.1 mmHg) prior to laparotomy compared to animals that did not receive xylazine. With the exception of a transient pulse pressure increase immediately after ischemia in the xylazine treated animals, the pulse pressures and heart rates continued unchanged for at least the first hour of ischemia (this point is illustrated in Figure 4 though it splits the animals into FFPD and SFPD animals).

In contrast to xylazine, glucose in the intestinal lumen had little effect on pre-ischemic MABP values (time = 0 in Figures 2 and 3), possibly because it had less time to act. However, it did decrease pre-ischemic pulse pressure (48.1 ± 8.3 mmHg for *Saline* vs. 40.3 ± 6.5 mmHg for *Glucose*, $P < 0.015$; 39.0 ± 7.2 mmHg for *Xyl Saline* vs. 34.0 ± 3.9 mmHg for *Xyl Glucose*, NS, $P = 0.066$). It also tended to decrease heart rate (in the absence of the stronger effect of xylazine; 359 ± 48 bpm for *Saline* vs. 321 ± 52 bpm for *Glucose*, NS, $P = 0.075$).

In some animals we measured blood glucose levels prior to ischemia and at time of death. Xylazine had a powerful effect on blood glucose levels (pre-ischemic values: 401 ± 53 mg/dl for *Xyl* animals vs. 122 ± 15 mg/dl for animals without xylazine; $N = 17$ and 13 respectively, $P < 1.4 \times 10^{-14}$) in agreement with reports in the literature that xylazine, as a parasympathetic alpha-2 adrenergic receptor agonist, inhibits pancreatic insulin release causing hyperglycemia (14). Interestingly, the effect of xylazine appears to be greater than having glucose or saline in the lumen of the intestine as inferred from time of death blood glucose values (55 ± 33 mg/dl for *Saline* and 115 ± 60 mg/dl for *Glucose* animals and 135 ± 113 mg/dl for *Xyl Saline* and 277 ± 163 mg/dl for *Xyl Glucose* animals). It is one reason we included animals without xylazine in this study.

Hemodynamic effects of ischemia

As expected, given the ability of gut ischemia to activate the sympathetic nervous system (9), *all* groups responded to ischemia with an immediate and pronounced increase in blood pressure (Figures 2, 3, 9, 10, and 11). The four non-treatment groups increased their blood pressure to approximately the same pressure upon arterial occlusion (Figures 2 and 3).

Parasympathetic “stimulation” lowers blood pressures and may increase incidence of fast fatal pressure drops

Animals in the *Xyl Glucose* group had a significantly larger drop in MABP during their *initial drop period* than animals in the *Saline* group and their MABP stayed significantly lower at many points throughout their pressure histories prior to their fatal pressure drops (*Xyl Glucose* shown in Figure 2 vs. *Saline* in Figure 3). Also, we saw that *Glucose* animals with SFPD had a lower MABP than *Saline* animals with SFPD at the time their fatal pressure drop started (Figure 3 **bottom**), at least in the absence of xylazine, which presumably masks the effect with its more powerful depressing effect on MABP (MABP was also lower in the *Glucose* group at the end of the initial rise and the beginning and end of the initial drop, but not significantly; $P=0.08$ for all three points). Though neither xylazine nor luminal glucose is a simple “stimulator” of the parasympathetic system, the depression of MABP seen here supports the reports that glucose and xylazine can activate the parasympathetic nervous system (10, 11, 13, 15).

In preliminary studies with xylazine and 2 hr SAO, we observed rapid pressure drops and subsequent death within minutes prior to the end of the two hour observation period in animals with glucose added to the intestinal lumen, but not with saline alone. This observation led to the hypothesis that an autonomic mechanism may be active in SAO and the decision to compare animals with glucose or saline in the intestinal lumen. Similarly, of the 10 animals in the *Xyl Saline* group, only 2 animals had a FFPD as compared to 7 of 14 FFPDs in the *Xyl Glucose* group (not significant, $P=0.065$). This difference is significant ($P<0.02$) if we include only the first 2 hrs of ischemia (as in the pilot study), since only 1 of the 2 *Xyl Saline* FFPDs occurred within the 2 hr time point as compared to all 7 of the *Xyl Glucose* FFPDs. Note that the fatal pressure drops of *Xyl Glucose* animals, in general, occurred sooner than that of *Xyl Saline* animals (90 ± 40 min for *Xyl Glucose* vs. 147 ± 43 min for *Xyl Saline*, $P<0.004$, when pooling FFPD and SFPD animals).

In the absence of xylazine, glucose in the lumen of the intestine had no apparent effect on FFPD incidence with 30% of the animals in the *Glucose* group and 33.3% of animals in the *Saline* group experiencing a FFPD within 4 hours of SAO (Table 2, the *Saline* group was not significantly different from the *Xyl Glucose* group).

Given the powerful hyperglycemia caused by xylazine and the fact that FFPDs occurred both in its presence and absence, we saw no correlation between blood glucose levels prior to ischemia and the incidence of a FFPD (correlation coefficient of -0.097).

To determine if intestinal distension is necessary for FFPDs to occur, we also subjected an animal to SAO without xylazine and without any injection directly into the intestinal lumen. The animal had a FFPD, indicating that FFPDs are possible even without fluid in the intestinal lumen.

Gross morphological organ damage

Animals subjected to SAO, but not shams, had damaged small intestines with extensive hemorrhagic lesions (Figure 5). The extent of hemorrhage increased with prolonged survival time, i.e. animals that expired rapidly had minimal damage to their intestines, while animals that survived for the duration or died near the end of the 4 hours showed extensive damage. This damage, visible to the unaided eye, was reduced with glucose in the lumen. In most SAO, but not sham animals, the pancreas developed hemorrhagic lesions and the liver had large pale areas reflective of a lack of red blood flow out of the intestine through the portal vein.

16 of the 25 animals with a FFPD, but only 1 of the 41 animals with a SFPD, exhibited a post-mortem gastric reflux (as assessed by the presence of a pool of yellow liquid forming by the head as fluid exited the mouth and nostrils; typically ~ 0.5 ml) within a few minutes of death ($P < 2.0 \times 10^{-8}$ by chi-square test). Gastric reflux was the only event we saw by unaided inspection tied almost exclusively to FFPDs. The single SFPD animal with a post-mortem gastric reflux was in the *Glucose ANG* group and was one of the 2 animals that survived the four hours with no sign of a fatal pressure drop. In that case, reflux occurred after euthanasia.

Animals with FFPDs have earlier fatal pressure drops and death than animals with SFPDs

Out of 77 rats subjected to SAO, all but 2 animals had a fatal pressure drop start within 4 hours of the onset of ischemia. By pooling their onset times, we saw that animals undergoing a FFPD initiated a fatal pressure drop significantly sooner than those with a SFPD (86 ± 47 min, $N=25$, for FFPD vs. 140 ± 40 min, $N=49$, for SFPD; $P < 1.5 \times 10^{-5}$). Animals with a FFPD also had significantly earlier death than animals with a SFPD (91 ± 48 min vs. 241 ± 45 min; $P < 3.7 \times 10^{-17}$). Excluding the *Xyl Saline* group, which did not have a sufficient number of FFPDs ($N=2$) to test the issue, this earlier death with FFPD remained statistically supported within the groups as well.

The FFPDs

The onset of the FFPDs often occurred within a single heartbeat (Figure 6). MABPs were already significantly lower within 10 seconds after the onset of the FFPD and decreased further over the next minute (Figure 7 **top left**). Pulse pressure, in contrast, did not drop until the next minute ($P < 0.05$) when the animal was already near death. Even then, the pulse pressure drop was proportionately less than the MABP drop (Fig. 7, middle left). Heart rate was reduced in the first 10 seconds ($P < 0.05$) but over the next minute did not drop further (Figure 7 **bottom left**), and in fact, the heart often continued to beat after no pulse was detectable in the arterial catheter (not the case with SFPD animals). The relatively small loss of heart rate and pulse pressure compared to MABP suggested the MABP reduction was due more to reduction of venous return than to loss of cardiac function.

We examined whether the pressure history may serve to identify animals at risk for a FFPD. The FFPDs were not predicted using MABP, pulse pressure, or heart rate prior to laparotomy, at time zero (just prior to ischemia), at the time of the peak MABP, or 20, 40, and 60 min after ischemia (pooling *Xyl* and *NoXyl* animals separately) (Figure 4). Neither did pressures in the Initial rise and Initial drop period differ in FFPD animals versus SFPD animals (Figures 2 and 3 top versus bottom rows).

We occasionally saw sharp drops and recoveries prior to the fatal drop, in some cases of a magnitude similar to that of the fatal pressure drops (Figure 1). We wished to determine if their incidence, magnitude, or other characteristics of the time between the end of the initial drop and the start of the fatal drop could predict a FFPD. Therefore, every line segment in this time period was classified, based on its slope, as a *stable segment*, *fast drop*, *fast recovery*, *slow drop*, or *slow recovery* (see Supplemental Table 1 for cut-off values). For each animal group with fast and slow fatal pressure drops (Supplemental Table 1), or pooled FFPD and SFPD animals (Supplemental Table 2), we determined within this period: 1) the number of line segments falling into each category, 2) the percentage of time spent in each category, and 3) the segments' average durations and 4) pressure changes, in order to determine, among other things, whether the fast drops of animals that went on to have a FFPD were more frequent or of greater magnitude than those with a SFPD.

Comparing animal groups (Supplemental Table 1), we saw that animals without xylazine had shorter stable line segments than *Xyl* animals (ANGD reversed this). Additionally, all pressure drops and recoveries were more frequent, and the slopes of the fast drops and fast recoveries were greater in magnitude, in the *Saline* group than in the *Xyl Glucose* group (reversed somewhat in the *Glucose* group and more so in the ANGD group). These were indicative of numerous high-frequency pressure fluctuations in the *Saline* group and is likely a sign of sympathetic stimulation not buffered by parasympathetic activity (8).

When animals were grouped according to the presence of FFPD or SFPD (Supplemental Table 2), we saw that: 1) Animals that went on to a FFPD had *fewer* fast pressure drops and recoveries than SFPD animals prior to the fatal pressure drop. Moreover, the slopes of fast drops and recoveries were lesser in magnitude in FFPD animals than SFPD animals, though the FFPD animals had greater pressure changes. 2) In the absence of xylazine, even slowly dropping/recovering segments were more frequent in the SFPD animals than in the FFPD animals. 3) SFPD animals have more stable segments than FFPD animals.

In summary, pressure fluctuated more in animals that went on to have a SFPD. Based on this particular analysis in the rat, the pressure history is not suitable for predicting individuals at risk for a FFPD in SAO.

The SFPDs

We could only determine the starting point of SFPDs to within about a minute (as opposed to a second in the FFPD groups). When comparing 5 min before vs. 5 min after the approximate beginning of the SFPD, we saw a significant drop in mean arterial blood pressure and a significant increase in heart rate (Figure 6 **right**). Pulse pressure did not change in this time.

The time of death from SFPD did not vary significantly between groups regardless of the presence of xylazine or glucose.

Treatments

ANGD prevented changes in plasma protease activity and reduced intestinal damage but did not prevent FFPDs

When we examined plasma protease activity in our 4 non-treatment SAO groups, we saw that the change in protease activity in animals with a SFPD was significantly greater at the time of collection (death or 4 hours ischemia) than in FFPD animals, which exhibited no significant change in plasma protease activity (-0.09 ± 1.67 Relative Fluorescent Units [RFUs] for FFPD animals [N=11] vs. 4.83 ± 2.85 RFUs for SFPD animals [N=16]; $P < 7.7 \times 10^{-6}$). This increase was likely due to the increased duration of ischemia, as time of collection correlated well with change in protease activity (Figure 8; correlation coefficient = 0.76; FFPD time of collection = 86 ± 47 min; SFPD time of collection = 226 ± 22 min).

ANGD treatment almost completely prevented visible signs of damage to the intestine, even after 4 hours, suggesting successful inhibition of luminal digestive enzymes. However, ANG D had no significant effect on the change in plasma protease activity other than a small increase in FFPD animals ($P < 0.027$) that may also be due to a later time of collection (1.71 ± 1.40 RFUs, 105 ± 65 min, N=7 for ANG D FFPD animals and 2.60 ± 2.28 RFU, 240 ± 0 min, N=4 for ANG D SFPD animals).

ANGD treatment, despite the absence of xylazine, increased the rate of death by FFPD to 67% (not significant, $P = 0.04$, Table 2), though it had no effect on the hemodynamics of the

FFPD animals (Figure 9 **top**). However, ANG2 significantly delayed the onset of SFPDs (Figure 9 **bottom**).

Parasympathetic blockade prevents fast fatal pressure drops

To determine whether interference with the parasympathetic nervous system may affect the incidence of FFPDs, we performed a total subdiaphragmatic vagotomy, or alternatively added glycopyrrolate, to SAO animals treated with xylazine and glucose to increase their incidence in FFPDs. Both vagotomy (*Xyl TSV Glucose*) and treatment with glycopyrrolate (*Xyl Gly Glucose*) prevented the FFPDs ($P<0.008$; Table 2).

Total Subdiaphragmatic Vagotomy had no effect on pressure history other than to prevent all FFPDs, as seen by the almost complete overlap of the *Xyl TSV Glucose* pressure history with that of the *Xyl Glucose* SFPD subset pressure history (Figure 11). Glycopyrrolate, in contrast, increased MABP even prior to ischemia (80 ± 8 mmHg vs. 67 ± 15 mmHg in the pooled *Xyl Glucose* FFPD and SFPD animals, $P<0.019$), yielded a higher pressure than any other group at the end of the initial rise, and delayed the onset of the large initial drop caused by xylazine, suggesting that it inhibited the parasympathetic nervous system (Figure 10). The difference in early hemodynamic effects between the two treatments may be because glycopyrrolate may have more effect on the peripheral vasculature than the subdiaphragmatic vagotomy. However, like the vagotomy group, the later events in the *Xyl Gly Glucose* group mirrored that of the *Xyl Glucose* SFPD animals, suggesting that the most prominent effect of the glycopyrrolate at later time points was to prevent FFPDs. Glycopyrrolate had no apparent effect on gross intestinal morphology.

Discussion

The current results in an acute model of severe gut ischemia by SAO show that systemic blood pressure first increases in response to ischemia then decreases past its initial value, ending in death. The fall in blood pressure may either be very rapid (occurring over seconds) or slow (over hours). Neither blood pressure/heart rate histories nor the prior existence of sharp drops and recoveries predicted rapid fatal pressure drops. Intramuscular xylazine and glucose in the lumen of the intestine, activators of the parasympathetic nervous system, increase the incidence of fast fatal pressure drops, while total subdiaphragmatic vagotomy or addition of glycopyrrolate, a parasympathetic inhibitor, prevent them. Digestive enzyme inhibition prevents neither fast nor slow fatal pressure drops, though it delays the slow ones.

Evidence contradicting a hypovolemic mechanism

We were concerned that some of the hypotension we observed in FFPDs or SFPDs could be attributed to hypovolemia from loss of fluid through the intestinal wall. However, pilot studies showed no change of weight in the intestine for hours after ligation of the superior mesenteric and celiac arteries. While glucose in the intestinal lumen would increase its osmolarity and thus its potential for pulling fluid from the vasculature, glucose did not increase the incidence of FFPDs in the absence of xylazine. Nor did glucose change survival time for SFPD animals. These results suggest the osmotic effects of glucose did not play a major role for FFPDs or SFPDs, and thus it is less likely that hypovolemia contributed to the hypotension.

Evidence supporting a neurogenic mechanism

There were a number of characteristics associated with FFPDs to suggest a neurogenic shock mechanism for pressure loss as opposed to a mechanism involving plasma mediators. First, we reduced mediator communication from the intestine by total obstruction of the two major arteries feeding the organ, occlusion of the major lymphatic vessel exiting the

intestine, and retention of the intestine outside of the body to prevent mediator release into the peritoneal space. Second, in hemorrhagic shock, which is affected by mediators from the intestine (16), hypotension is typically accompanied by tachycardia (17). Instead we observed either a transient bradycardia or normocardia accompanying the FFPDs, a trait of neurogenic shock (18). Third, pulse pressure was preserved or dropped only to a small degree after a FFPD until the animals were near death. The heart muscle visibly continued to contract after FFPD but not SFPD, even at zero femoral arterial blood pressure and pulse pressure, which, combined with normocardia, suggests that pressure loss was due less to cardiac failure, and more to a failure of peripheral resistance and venous return. Fourth, we observed, often within a single heartbeat, an immediate transition in the blood pressure slope at the start of fast fatal drops. This short time interval suggests a neurogenic event as opposed to an effect from an evolving circulating inflammatory mediator. Fifth, FFPDs were not prevented by protease inhibition in the intestinal lumen, which has been shown to improve survival and reduce the presence of shock mediators in the plasma of SAO with reperfusion animals (1, 3, 4). Sixth, gut ischemia is known to activate the sympathetic nervous system (9), and we observed a powerful and near immediate increase in MABP upon SAO, suggesting this is also present in our model. Seventh, activators of the parasympathetic nervous system tended to increase the incidence of FFPDs, while parasympathetic nervous system disruption prevented them. Finally, we observed after most of the FFPDs, and almost never after SFPDs, a post-mortem gastric reflux. Since loss of sympathetic stimulation results in increased gastric tone (19) and the lower esophageal sphincter is relaxed by parasympathetic stimulation (20), gastric reflux is also in line with an autonomic nervous system failure.

Though operating by a different mechanism than FFPDs, SFPDs may also have a neurogenic component. SFPDs animals had 1) restricted mediator release from the intestine, 2) sympathetic nervous system activated by SAO, 3) normocardia after SFPD onset, 4) preserved pulse pressure despite pressure loss, and 5) were not prevented by protease inhibition. However, death from SFPD occurred at approximately the same time regardless of xylazine, glucose, TSV, or glycylpyrrolate, suggesting independence from the parasympathetic nervous system. Thus, the SFPDs resemble traditional neurogenic shock, which only requires dysfunction of the sympathetic nervous system.

Neurogenic Shock

Classical neurogenic shock is caused by a failure of the sympathetic nervous system to maintain vascular tone. Vasodilation decreases venous return as blood pools in the periphery leading to decreased cardiac output. Neurogenic shock has mostly been reported to be associated with brain and spinal cord injury (12). Acute spinal cord injury, for example, can cause an explosive sympathetic discharge, followed by failure. However, neurogenic shock has also been associated with hypovolemia (21), emotional shock (22), burns (23), and gastric rupture (24). Recent results in rats show that administration of endotoxins produced by *Bacillus anthracis*, which produces shock-like cardiovascular alterations, first increases and then leads to a decrease in sympathetic nerve discharge and death at about the same time as our SFPD animals (25). The current results suggest for the first time neurogenic shock originating from severe gut ischemia.

The combined evidence suggests that prolonged, increased sympathetic discharge (whether from ischemia, spinal cord injury, or infection by *Bacillus anthracis*) is not sustainable and can result in death, as the sympathetic nervous system ceases to function over the following hours when either the brain fails to send out sympathetic efferent signals or the blood vessels fail to respond to such signals. This hypothesis is in line with reports that one cause of the progressive hypotension in hemorrhagic shock is vascular hyporeactivity to norepinephrine, etc., that develops due to hyperpolarization of arterial smooth muscle cells via changes in

ion channel function (26). To determine whether the SFPDs occur by the same mechanism as traditional neurogenic shock, direct measurements of sympathetic and/or parasympathetic nerve activity and other additional experiments will be required.

Current models for neurogenic shock in the literature are related only to spinal cord or brain injuries or direct nerve and/or brain stimulation (PubMed search with keywords “neurogenic shock”). If direct nerve activity measurements confirm a neurogenic shock, gut ischemia may also serve as a useful model for other, more difficult to model, forms of neurogenic shock resulting from sustained sympathetic stimulation (e.g. emotion-triggered neurogenic shock).

Relevance to hemorrhagic shock

SAO followed by reperfusion is a model for hemorrhagic/hypovolemic shock with release of shock mediators from an ischemic gut region into the central circulation, as the gut may be ultimately responsible for death (16). However, if neurogenic shock is a cause of death in gut ischemia, a neurogenic mechanism may also be at least partially responsible for blood pressure loss in clinical cases of hemorrhagic shock or trauma. Supporting this hypothesis are studies showing that hemorrhagic shock activates the sympathetic nervous system (27), and septic shock is accompanied by loss of vasomotor tone and cardiovascular depression (28).

Additionally, we observed animals in a 2 hr hemorrhagic model of shock spontaneously die prior to reperfusion after a rapid pressure loss that did not respond to fluid resuscitation (2). This evidence suggests that FFPDs may also occur in hemorrhagic shock, though future studies will be required to determine whether the same mechanism is involved. If it is involved, then prophylactic parasympathetic inhibition, such as with glycopyrrolate, may be indicated in conditions in which resuscitation options are limited (battlefield remote locations, surgical requirements, etc.) leading to prolonged ischemia, though bleeding with increased pressure could remain a problem.

Role of parasympathetic nervous system in SAO

Both fast and slow fatal pressure drops may occur as a result of a large-scale vasodilation. SFPDs may occur due to a gradual failure of the sympathetic nervous system that may be irreversible as long as the trigger or need for an elevated sustained sympathetic nerve discharge remains. FFPDs may occur due to short-term decreases in the sympathetic output, which may become less stable when the sympathetic nervous system is stimulated by ischemia (e.g. the high-frequency fluctuations we observe in the absence of strong parasympathetic buffering by xylazine/glucose, Supplemental Table 1). Our results with glycopyrrolate and total subdiaphragmatic vagotomy suggest that what separates a fatal fast drop in blood pressure from one that recovers is the degree of parasympathetic activity. Strong parasympathetic nervous system stimulation uncompensated by sympathetic nervous system activity, however brief, can cause blood pressure to drop below recoverable levels. This possibility is supported by the trend towards increasing incidence of FFPDs with the parasympathetic nervous system activators, xylazine and glucose.

Protease inhibition with ANG2

ANG2 in the lumen of the intestine attenuates the enzymatic destruction of the mucosal barrier and entry of digestive enzymes into the wall of the intestine (4). We saw here that it preserved gross intestinal morphology. Interestingly, ANG2 in the intestinal lumen did not significantly prevent the increase in plasma protease activity with prolonged intestinal ischemia, suggesting that the proteases in the blood may not all be derived from the

intestinal lumen. This makes sense given that one goal of our surgical procedure was to prevent entry of mediators generated in the intestine into the rest of the circulation.

ANGD delayed the onset of SFPDs and may delay death during SFPDs (though a longer observation period might be required to determine the latter). One possible explanation for this observation is that, by preserving the morphology of the intestine, ANG D may attenuate the enzymatic destruction of nerve fibers such as sympathetic afferents in the wall of the intestine, which could then continue to detect and report gut ischemia, stimulating a longer pressure increase before failure. An intact intestine may also provide continued nutrition and thus maintain sympathetic nervous system function.

ANGD tended to increase the incidence of FFPDs (not significant, $P=0.04$) without apparently altering the FFPD animals' pressure histories. This might also be due to protection of nerve fibers or even a more direct action on the autonomic nervous system promoting instability of sympathetic nervous system output or increasing parasympathetic activity over time. This hypothesis is suggested by the fact that ANG D tended to mimic the effects of xylazine in the period between the initial and fatal drops (Supplemental Table 1) and by pilot studies with SAO and intravenous ANG D which had depressed MABP in comparison to animals with SAO and no intravenous ANG D (not shown). Interestingly, we recently observed a rat given both intramuscular xylazine and intraluminal glucose/ANGD experience a fast drop in blood pressure *even without ischemia*, suggesting a parasympathetic stimulus may cause a FFPD even without increased sympathetic activity.

Implications of FFPDs

Given the relatively short time interval between the onset of ischemia and death, a fast fatal drop in humans may not always be preventable by conventional resuscitation. Moreover, death would not necessarily be attributed to neurogenic shock and/or gut ischemia since the fast form of death in our model leaves little evidence behind for the actual cause of death. Therefore it may be difficult to conclude with certainty what proportion, if any, of human deaths are due to a FFPD in the absence of continuous blood pressure monitoring. In the case of hospital patients with severe gut ischemia (e.g. due to a blood clot) or cases of prolonged hypotension, or when resuscitation is not possible, the current results suggest that analysis of the incidence of FFPD in humans is needed and treatment with a parasympathetic inhibitor such as glycopyrrolate or atropine, may be tested to minimize the incidence of FFPDs, if they occur.

The current model may also be relevant for analysis of Sudden Infant Death Syndrome (SIDS) or Sudden Cardiac Death (SCD). SIDS remains unexplained after forensic autopsies and detailed death scene investigations (29). Currently the only significant extrinsic risk factor for SIDS is infant sleep position, which affects vasomotor tone (30). SIDS has been linked to autonomic dysregulation (31), death can occur in under 20 minutes (32), there is intestinal damage (33), and gastric reflux in 30 to 40% of cases (34), all characteristics shared by our model.

In Sudden Cardiac Death, death is frequently assumed to be the result of an arrhythmia. However, some of the same triggers of arrhythmia, namely ischemia and sympathetic activation (35), are triggers for the mechanism of sudden death we describe here. Given that most SCD occur outside of the hospital and are resistant to cardiopulmonary resuscitation (35), it is possible that in many cases the cause of death may not have been due as much to cardiac failure as to an autonomic failure leading to vessel dilation and loss of venous return.

Though the effects of glucose, xylazine, and ANG D are not completely understood, their use in combination with SAO, or other forms of sympathetic stimulation, may prove useful for

the study of Sudden Infant Death Syndrome, Sudden Cardiac Death, or other forms of sudden death, by creating a reproducible form of sudden death that occurs with high incidence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ANGD	6-amidino-2-naphthyl p-guanidinobenzoate dimethanesulfonate
FFPD	fast fatal pressure drop
MABP	mean arterial blood pressure
SAO	splanchnic arterial occlusion
SCD	sudden cardiac death
SIDS	sudden infant death syndrome
SFPD	slow fatal pressure drop
TSV	total subdiaphragmatic vagotomy

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Figure 1.

Typical blood pressure histories of four non-treatment SAO animal groups with their approximated pressure history superimposed (open squares). The *upper left* and *middle right* histories show fast fatal pressure drops (FFPDs) as defined by the slope of the fatal pressure drop. In contrast, the *upper right* and *middle left* histories demonstrate slow fatal pressure drops (SFPDs). Other features include the large, concave initial drop seen in most xylazine treated animals (in contrast to the short, linear initial drops of the animals that did not receive xylazine), the high frequency fluctuations around the trend-line often observed in animals that did not receive xylazine, and several large, sharp pressure drops similar to FFPDs but with recovery of the pressure. The *bottom* history is a 6 min excerpt from the *upper right* history showing the high frequency fluctuations in greater detail and the corresponding trend-line placement.

Figure 2.

Blood pressure histories of the Xyl Saline and Xyl Glucose groups of SAO animals constructed from the means $\pm$ SD of the starting times, starting pressures, durations, and pressure changes of their *initial rise periods*, *initial drop periods*, and *fatal drop periods*, as well as the time of death. *Top row*: pressure histories of animals with a fast fatal pressure drop (FFPD). *Bottom row*: pressure histories of animals with a slow fatal pressure drop (SFPD). * denotes a significant ($P < 0.025$) difference in the ending time of the initial drop, \$ denotes a significant difference in the duration of the initial drop. Note that 5 animals in the Xyl Saline group and 2 animals in the Xyl Glucose group clearly started a SFPD but did not die within the 4-hour observation period.

Figure 3.

Blood pressure histories during SAO of the Saline and Glucose groups constructed from the means $\pm$ SD of the starting times, starting pressures, durations, and pressure changes of their *initial rise periods*, *initial drop periods*, and *fatal drop periods*, as well as the time of death. *Top row*: pressure histories of only those animals with a fast fatal pressure drop (FFPD). *Bottom row*: pressure histories of only those animals with a slow fatal pressure drop (SFPD). # $P < 0.025$ vs. starting pressure of the fatal drop in the Glucose SFPD group. Note that 1 animal in the Saline group and 2 animals in the Glucose group clearly started a SFPD but did not die within the 4-hour observation period. Additionally, 1 animal in the Saline group did not show any fatal pressure drop in the observation time and was therefore excluded from calculations of variable zone duration, start and ending points of the fatal drop and time of death. Statistical comparisons were also made between the Saline group shown here and the Xyl Glucose group in Figure 2 (i.e. minimal vs. maximal treatment with parasympathetic “stimulators”), but to preserve clarity are not shown by symbols. Statistically significant differences for the FFPD animals were as follows: The Saline group started from a higher pressure, but had less of a pressure increase and a shallower slope in its

initial rise than Xyl Glucose group (i.e. started from a higher pressure, but ended at around the same pressure in the same amount of time). Additionally, the initial drop period of the Saline group had less of a pressure drop and ended at a higher pressure than the initial drop of the Xyl Glucose group. The SFPD animals (*bottom rows*) had all of the significant differences of the FFPD, *plus* the following: The initial drop of the Saline group was also of a shorter duration and ended at an earlier time point than that of the Xyl Glucose group. The amount of time between the end of the initial drop and the start of the fatal drop was also greater for the Saline group and the fatal drop started at an earlier time point and from a higher pressure. However, the duration of the SFPD was less in the Saline group than in the Xyl Glucose group so that there was ultimately no difference in time of death.

Figure 4.

Arterial blood pressure (MABP; *top*), pulse pressure (*middle*), and heart rate (*bottom*) prior to laparotomy (Pre-lap), immediately before ischemia, at peak MABP, and at 20, 40, and 60 minutes post-occlusion for the Xyl Saline and Xyl Glucose (*left*) and Saline and Glucose groups. The values are separated according to fast (FFPD) or slow fatal pressure drop (SFPD). Some animals had a FFPD at a time before 40 or 60 min and are excluded from those time points. * $P < 0.05$ vs. FFPD animals. Arrow indicates the only point at which the MABP of the xylazine treated animals was *not* significantly different from those without xylazine (all heart rates and pulse pressures were significantly different between those with and those without xylazine).

Figure 5.

Representative images of the small intestine after sham surgery (*top left*) or SAO leading to a fast (*bottom left*) or slow (*bottom right*) fatal pressure drop. Note that the fast fatal pressure drop animal (time of death = 39 min) has a relatively undamaged intestine while the slow fatal pressure drop animal, which survived through the end of the 4 hr ischemic period, has multiple areas of hemorrhage (the dark purplish-red areas), with blood accumulation around Peyer's patches (arrows). Also shown are representative images of the effects of SAO on liver (appearance of pale regions, *upper right*) and pancreas (hemorrhages, *middle right*). Bar = 1 inch.

Figure 6.

Representative trace of arterial blood pressure during a 15 second period that includes the onset of the fast fatal pressure drop (arrow). In this particular case, heart rate dropped after the FFPD. In several cases heart rate remained constant.

Figure 7.

Left: Arterial blood pressure (*top*), pulse pressure (*middle*), and heart rate (*bottom*) of animals (pooled from all four non-treatment groups) with a fast fatal pressure drop (FFPD) immediately before, 10 seconds after, and 1 minute after the start of the FFPD (N = 17). * P<0.05 vs. immediately before the FFPD; # P<0.05 vs. 10 seconds after the FFPD.

Right: Mean arterial blood pressure (*top*), pulse pressure (*middle*), and heart rate (*bottom*) of animals (pooled from all four non-treatment groups) with a slow fatal pressure drop (SFPD) 5 min before and 5 min after the approximate start of the SFPD (N = 25). * P<0.05 vs. 5 min before SFPD.

Figure 8.

Change in plasma protease activity (initial plasma collected prior to laparotomy, final plasma collected at time of death or 4 hours) vs. the time after ischemia of collection of the final plasma. Trend-line is for all animals. Protease activity was measured by digestion of a fluorescent casein substrate. Correlation coefficient = 0.76.

Figure 9.

Blood pressure histories during SAO of the Glucose and Glucose ANG D groups constructed from the means $\pm$ SD of the starting times, starting pressures, durations, and pressure changes of their *initial rise periods*, *initial drop periods*, and *fatal drop periods*, as well as the time of death. Note this is the same data for the Glucose group shown in Figure 4 repeated here for convenience. *Top*: pressure histories of only those animals with a fast fatal pressure drop (FFPD). *Bottom*: pressure histories of only those animals with a slow fatal pressure drop (SFPD). * denotes significant difference in fatal pressure drop onset time ($P<0.025$).

Note that 3 animals in the Glucose ANG D group and 2 animals in the Glucose group started a SFPD but did not die within the 4-hour observation period. 1 animal in the Glucose ANG D group did not show any fatal pressure drop in the observation time and was excluded from calculations of duration of the period between the initial and fatal drops, the start and ending points of the fatal drop, and time of death.

Figure 10.

Blood pressure histories of animals (means $\pm$ SD of the starting times, starting pressures, durations, and changes in pressure of their *initial rises*, *initial drops*, and *fatal drops*, as well as the time of death) treated with glycopyrrolate vs. the control group with its FFPD and SFPD animals plotted separately. Note the control group data was shown in Figure 3 and is repeated here for convenience. * denotes a significant ($P < 0.025$) difference in the starting or ending time point of a period versus the equivalent event (e.g. start of the fatal drop) in *both* of the other histories, # denotes a significant differences in pressure, % denotes significant differences in slope, \$ denotes a significant difference in the period's duration. Additional significant differences not shown for clarity: The initial drop of the Glycopyrrolate group started significantly later than the initial drop of the Xyl Glucose SFPD group, and the amount of time between the end of the initial drop and the start of the fatal drop was significantly less in the Xyl Glucose FFPD animals than in the Xyl Glucose SFPD animals. Additionally, the slope of the period between the end of the fatal drop and death was significantly steeper in Xyl Glucose FFPD animals than in Xyl Glucose SFPD animals.

Figure 11.

Blood pressure histories of animals (means $\pm$ SD of the starting times, starting pressures, durations, and changes in pressure of their *initial rises*, *initial drops*, and *fatal drops*, as well as the time of death) treated with total sub-diaphragmatic vagotomy (TSV) vs. the control group with its FFPD and SFPD animals plotted separately. Note the control group data was shown in Figures 3 and is 10 repeated here for convenience. All significant differences shown are between Xyl Glucose FFPD and Xyl TSV Glucose groups (No significant differences between the TSV group and the Xyl Glucose SFPD group, and Xyl Glucose FFPD vs. Xyl Glucose SFPD differences are shown in Figure 10). * denote significant ($P < 0.025$) differences in the start or end time point of a period versus the equivalent event in the Xyl TSV Glucose group, \$ denote significant differences in period durations, % denotes a significant difference in period slope.

Table 1

Key characteristics of fatal pressure drops

	$T_{\text{death}} - T_{\text{Fatal Drop}}$ (min)	Slope of FPD (mmHg/min)
Fast Drop	4.49 ± 1.68 Range: 2.7 to 10.10	-75.86 ± 38.35 Range: -13.21 to -158.64
Slow Drop	103.25 ± 37.36 Range: 39.7 to 207.90	-0.84 ± 0.45 Range: -0.35 to -2.58

Footnote: Two animals had no obvious fatal drop (one in the Saline and one in the Glucose ANG D group) and one animal (in the Saline group) fell between the two fatal drop types. These cases were excluded from the above analysis. In the remaining calculations for this report, they were grouped with the SFPD animals.

Table 2

SAO animals grouped by type of fatal pressure drop

Experimental Group	Fast Fatal Pressure Drop	Slow Fatal Pressure Drop	% FFPD
Xyl Saline	2	8	20.0
Xyl Glucose	7	7	50.0
Saline	5	10	33.3
Glucose	3	7	30.0
Glucose ANG1	8	4	66.7
Xyl Gly Glucose	0*	8	0.0
Xyl TSV Glucose	0*	8	0.0

* P<0.008 by chi-squared test vs. Xyl Glucose group.

P=0.042 for Glucose ANG1 vs. Glucose

P=0.065 for Xyl Saline vs. Xyl Glucose.