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Baroreflex, heart period, and blood pressure characteristics in splanchnic artery occlusion induced shock

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Running Head: Blood pressure regulation in SAO shock

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Abstract

Continuous arterial blood pressure waveforms were collected in rats (n=26) during a protocol of shock induced by prolonged intestinal ischemia. Following splanchnic artery occlusion, arterial blood pressure progressively decayed until a fatal pressure drop, which could either be slow (SFPD) or fast (FFPD). In the latter case, the pattern of the arterial blood pressure decay led to cardiovascular collapse. In order to analyze the status of autonomic control of cardiovascular function during ischemia, time series of mean arterial and systolic blood pressures, and heart periods were derived from the arterial blood pressure records, and analyzed in the time domain and used for the computation of baroreflex sensitivity. Mean arterial pressure and heart period were also analyzed by the adaptation of the sample asymmetry based algorithm known as “Heart Rate Characteristics”, together with the Heart Period Characteristics and the Blood Pressure Characteristics indices.

Mean arterial pressure decreased in both SFPD and FFPD rats from the initiation of splanchnic artery occlusion to the fatal pressure drop, while heart period increased in FFPD rats (+18±27 ms), and decreased in SFPD rats (-19±27 ms, p-value < 0.01). Similarly, the baroreflex sensitivity in the low frequency band (0.25-0.75 Hz) increased in the FFPD rats (0.142 ± 0.072 ms/mmHg) and decreased in the SFPD rats (-0.053 ± 0.157, p-value < 0.01). As regards heart period characteristics, a lower value was found for FFPD rats vs. SFPD rats (0.76 ± 0.57 vs. 1.94 ± 1.27, p-value = 0.04), while a higher value was found for blood pressure characteristics (3.02 ± 2.87 vs.: 1.47 ± 1.29, p-value = 0.08). These results integrate previously reported data by a possible vagal mechanism for sudden death in FFPD animals, suggesting that sympathetic control could be overactivated and possibly reach saturation, as indicated by the heart period and baroreflex sensitivity. Finally, the heart period and blood pressure characteristics indices showed that they could have diagnostic value by predicting a higher risk for fatal hypotension.
INTRODUCTION

One of the most controversial problems in critical illness is the source of hemodynamic instability that threatens the life of acute patients. This instability is reflected by ample swings in arterial blood pressure (ABP), hypotensive crises and reduced tissue perfusion, and an irregular heart beat, that are difficult to manage with fluids, vasopressors and adrenergic agonists and antagonists. Little is known about the alterations of autonomic nervous system (ANS) function, and in particular on the baroreflex control of heart rate (HR) and arterial pressure in the presence of hemodynamic instability and cardiovascular (CV) dysfunction due to shock.

Therefore, the maintenance of cardiovascular stability in acute patients is a major clinical challenge for emergency and intensive care physicians. The effectiveness and timeliness of therapy, such as for example the interventions defined within the framework of the early goal directed therapy (32, 32) could rely on new, more powerful quantitative tools for the stratification of the risk, for the prediction of the outcome of the therapy against hemodynamic instability and hypotension, and for the evaluation of the recovery of the patient. However, such goal could be attained only if a more thorough description of the patho-physiology of autonomic regulation in shock is first achieved.

In order to gain insight into the impairment of ANS control in circulatory shock and its impact in the management of critically ill patients, new hypotheses on the relationship between the fundamental mechanisms of the disease and the dynamic features of hemodynamic signals need to be formulated and tested by means of appropriate experiments. Typical animal models of shock induced by trauma, hemorrhage, and sepsis (13, 24, 31) have shown important cardiovascular alterations and diffuse organ damage, due to low perfusion and hypotension, and independent of the initial insult.

Another important experimental model for the induction of shock is intestinal ischemia (15, 18, 22, 30), which can be achieved by ligating the splanchnic artery and generating splanchnic artery occlusion (SAO). In a report on the effects of SAO in rats, Penn and Schmid-Schönbein (30) showed that prolonged intestinal ischemia results in a steady, progressive and fatal decline in
arterial pressure and the possibility of sudden cardiovascular collapse. In this study, only the animals treated with vagolitic drugs or vagotomized did not experience sudden death, prompting the authors to propose the existence of a vagal mechanism for sudden death in intestinal ischemia induced shock.

The results of the mathematical analysis of the arterial pressure recordings from a subset of animals from the same database, aimed at investigating the impact of SAO shock on autonomic control of circulation, are reported in the present manuscript. The approaches applied to the selected arterial pressure records include a model to track baroreflex sensitivity (BRS) during the experiment, and the application of an algorithm developed by Moorman and coworkers (11, 21, 23, 28) and named Heart Rate Characteristics, which was readapted to the arterial pressure signal in order to describe the Blood Pressure Characteristics, as explained in Section II.

The specific objective of this paper is to investigate ANS control of heart rate during SAO induced shock (by means of BRS analysis), in order to further explore the vagal hypothesis for cardiovascular collapse, and to search for quantitative indices able to discriminate the risk of sudden cardiac death by means of the assessment of the dynamic features of arterial pressure and heart rate via computation of Blood Pressure Characteristics and Heart Period Characteristics.

II. MATERIALS AND METHODS

A. Experimental Protocol

An experimental study of circulatory shock was realized by means of SAO and intestinal ischemia was maintained for up to 4 hours or until the spontaneous death of the animal (31). The protocol was reviewed and approved by the University of California San Diego Animal Subjects committee, and the experiments were performed in adherence to the National Institutes of Health Guidelines on the Use of Laboratory Animals. The detailed description of the protocol is reported in (31).
Briefly, male Wistar rats were given general anesthesia with sodium pentobarbital, (50 mg/kg). A fluid filled arterial catheter connected to a pressure transducer was placed in the left femoral artery for continuous recordings of arterial pressure (sampling frequency $f_s = 1$ KHz); a venous line was inserted in the left femoral vein for medication and supplemental anesthesia. SAO was realized by ligation of the superior mesenteric and celiac arteries. The experimental groups were characterized by different treatments either into the intestinal lumen (i.l.), or via intravenous (i.v.) infusion or intramuscular (i.m.) injection (Table I).

Prior to general anesthesia, rats from groups C and D were tranquilized with an i.m. injection of xylazine (4 mg/kg). Following anesthesia and cannulation, the intestine was exposed and saline solution (group B) and glucose (100 mg/ml, groups A, C, D) were injected directly in the intestinal lumen. Group D also received i.v. glycopyrrolate (0.5 mg/kg). The ligation of the arteries was executed immediately upon completion of the i.l. injections, with the exception of group D, which was allowed to adjust to the response induced by the infusion of the i.v. glycopyrrolate for 15 minutes before SAO.

Following the ligation, no further intervention was performed and the animals were left undisturbed until spontaneous death (mean arterial pressure (MAP) < 20 mmHg and pulse pressure < 1 mmHg) or until 4 hours, when surviving animals were euthanized (120 mg/kg sodium pentobarbital, i.v.).

In (31), seventy-seven animals were divided into seven different groups. Only a subset of that database was included in our analysis. The justification for excluding several animals was the sampling frequency of the arterial pressure recordings (100 Hz) in fifty-one animals out of the original seventy-seven. The review by Bathia et al. (5) on the minimum sampling rate for the analysis of cardiovascular variability in rodents discussed thoroughly the evidence that arterial pressure waveforms need much higher fidelity in order to be analyzed mathematically, due to the fast heart rate of these species. Hence, only the rats from the four groups described above (ABP $f_s = 1$ KHz), were considered for this study (Table I).
Independently of the treatment received by the rats, the SAO experiment displayed two long-term
effects, from the standpoint of the ABP signal and outcome. Upon gut ischemia, mean arterial
pressure transiently rose (short-term response to clamping), and then dropped according to two
patterns: (i) gradually over ~ 1 to 3 hours (slow fatal pressure drop, SFPD) or (ii) rapidly (often by
more than 80 mmHg) over a period of 1 to 6 minutes (fast fatal pressure drop, FFPD), following an
initially slow decay. The mathematical analysis that is described in the following focused on the
physiological and statistical characterization of the differences between these two outcomes.

B. Arterial blood pressure preprocessing and analysis

Arterial pressure waveforms were processed by adapting the algorithm of Zong et al. 45) to the
heart rate of the rats, and the onset of the systole was identified first for each heartbeat, and then
used to search for the diastolic value within each cycle. Beat-by-beat series of systolic blood pressure (SBP),
diastolic blood pressure (DBP), and mean arterial pressure (calculated as the average of blood pressure over a cardiac cycle), were extracted from the pressure waveforms. The heart period (HP), i.e. the equivalent of the RR interval, was
assessed as the time interval between two consecutive diastolic values. The series were filtered to
eliminate ectopic beats and artifacts by means of an algorithm derived from (42), and were
resampled in the time domain, by application of an antialiasing filter, in order to obtain equally
spaced values over the chosen sampling period. The sampling frequency was set to 5 Hz, which is
considered a suitable frequency to capture the whole band of the cardiovascular spectrum in rats (5,
6, 16, 17).

Given the variability in the duration of the single experiments, it was necessary to standardize the
choice of the windows for the analysis of the series, in function of the overall goal, i.e. the
description of the features of ABP with respect to the outcome of the shock experiment (FFPD vs.
SFPD). For the purpose of this study, two periods were investigated: a) the period starting with
baseline recordings following anesthesia and cannulation and preceding any treatment and
ischemia, and ending 30’ after SAO; b) the period starting 30’ after SAO, that is immediately after the previous period, and lasting until the fatal pressure drop (FPD).

The rationale for this choice was to first assess the early changes from baseline induced by intestinal ischemia, taking into account the strong mechanical disturbance on blood pressure and hemodynamics caused by the ligation of the arteries and the sudden interruption of perfusion of the splanchnic region. The second period was isolated instead to describe the evolution towards a fatal decrease in pressure, which could be either sudden (FFPD) or progressive (SFPD). It was assumed that the early effects of ischemia take place in the first 30’ after SAO, and shock symptoms follow thereafter. This assumption was based on evidence from experimental models of circulatory shock, which led to a severe injury of the intestinal walls (7, 8, 12, 15, 18, 31, 38, 39). Such insult is instrumental in unleashing a powerful inflammatory response that consists of extremely harmful mechanisms, such as the autodigestion by the digestive enzymes (7, 8, 12, 15, 18, 31, 38, 39), and the general leakage of pro-inflammatory and injurious factors that can reach the systemic circulation via the portal vein system, the intestinal veins, and the lymphatics (9), playing a fundamental role in the progressive evolution towards multi-organ failure and death due to shock.

C. Time domain analysis

The arterial pressure and heart period time series were first analyzed in the time domain by calculating the following indices in the four treatment groups (A-D):
- the mean values over a stationary, 1 minute long window immediately before SAO;
- the maximum and minimum values of MAP and of HP after the beginning of SAO, and following the spike in ABP caused by the ligation of the mesenteric and celiac arteries (Figure 1);
- the mean values over a stationary, 1 minute long window at the end of the 30’ interval identified after SAO initiation.

As regards the interval from 30’ after SAO until FPD (either FFPD and SFPD), the mean values of arterial pressure and heart period series were calculated for two stationary windows at the beginning of this period, and at the end of it, before FPD. For this second window of observation, the rats were divided according to the final outcome of the experiment, i.e. groups FFPD and SFPD.
Moorman and coworkers (11, 21, 23, 28) proposed to compute the Sample Asymmetry (SA) of the distribution of the differences between the duration of RR intervals and the median of the RR series as a way to discriminate the effects of disease or experimental challenges on the distribution of heart rate values. This approach was an adaptation of a previous algorithm by Kovatchev et al. (20), and generates a statistical index, which was referred to as Heart Rate Characteristics, which was found to have predictive and diagnostic value (11, 23).

In this manuscript, this calculation was applied both to the heart period and mean arterial pressure series, and for this reason we refer to the two indices as Heart Period Characteristics (HPC) and Blood Pressure Characteristics (BPC).

Given $n$ samples of a series $\{x_i\}$, this approach consists in computing the quadratic function $r$:

$$ r(x_i) = (x_i - m)^2 $$  \hspace{1cm} (1)

where $m$ is the median of a distribution.

The amplitudes of the two branches of the quadratic function $r$ are characterized by two parameters ($R_1$ and $R_2$, see eqs. 2, 3), which quantify the deviation from the median, and in particular the prevalence of beat-to-beat increments or decrements of the considered variable. In other words, in the case of the Heart Rate Characteristics or equivalently the Heart Period Characteristics, it is possible to assess the prevalence of accelerations or decelerations of the heart rhythm within the considered window; in the case of the Blood Pressure Characteristics, the predominance of episodes of hypotension or hypertension or simply restoration of mean arterial pressure following hypotensive or hypertensive episodes.

$$ R_1 = \frac{1}{n} \sum_{i} r^2(x_i) \text{, with } r(x_i) = 0 \text{ for } x_i < m $$  \hspace{1cm} (2)

$$ R_2 = \frac{1}{n} \sum_{i} r^2(x_i) \text{, with } r(x_i) = 0 \text{ for } x_i > m $$  \hspace{1cm} (3)

The sample asymmetry is then defined as the ratio between $R_1$ and $R_2$:

$$ SA = R_1/R_2 $$  \hspace{1cm} (4)
An evenly distributed function $r$ is characterized by a value of SA close to 1. A skewed distribution of the function $r$ is characterized by values of SA either $< 1$ or $> 1$. In the former case, the technique detects the prevalence of increases in the heartbeat duration (in the case of HPC) and of reductions in arterial pressure (in the case of the BPC). In the latter case, the method detects respectively the prevalence of decrements in the duration of heart period and increments in blood pressure.

HPC and BPC were calculated during the late period of the protocol (i.e., from 30’ after SAO until FPD), with the goal of identifying differences between the SFPD and FFPD groups: a) over the entire duration of the interval since from 30’ after SAO to FPD; b) over stationary, artifact free, 3’ long, 50% overlapped running windows across the entire interval.

**E. Baroreflex sensitivity**

Baroreflex control of heart period was investigated by estimating BRS. A bivariate model that considers the causal relationship from systolic blood pressure to heart period, i.e. the feedback (FB) mechanism, and HP to SBP, i.e. the feed forward (FF) mechanism (10, 43) was identified. The effects of changes in SBP on HP account for the beat-by-beat regulation of HP by the cardiac baroreflex, i.e. the actual FB mechanism, whereas the effects of changes in HP on SBP represent the direct influence of heart cycle duration on blood pressure, which is not mediated by autonomic control, but instead by a perturbation mechanism based on Starling’s law (43).

Prior to estimating the coefficients of the model, the HP and SBP series were detrended by an order 10 interpolating curve. Then, an autoregressive bivariate model of order $p=8$ was computed:

$$Y[n] = \sum_{k=1}^{8} A_k Y[n-k] + W[n]$$ (5),

where:

$$A[k] = \begin{bmatrix} a_{11}[k] & a_{12}[k] \end{bmatrix} \begin{bmatrix} Y[n] \end{bmatrix} + \begin{bmatrix} a_{21}[k] & a_{22}[k] \end{bmatrix} \begin{bmatrix} Y[n] \end{bmatrix} = \begin{bmatrix} \mathcal{H}[n] \end{bmatrix} + \begin{bmatrix} \mathcal{W}[n] \end{bmatrix} = \begin{bmatrix} \mathcal{W}_{HP}[n] \end{bmatrix} + \begin{bmatrix} \mathcal{W}_{SBP}[n] \end{bmatrix}$$ (6).

The coefficients $a_{ij}$ were then used to calculate the gains of the transfer functions, according to (43):
The coherence between the HP and SBP series was assessed, with the maximum value searched in the low frequency (LF, 0.25 Hz < f ≤ 0.75 Hz) and high frequency bands (HF, 0.75 Hz < f ≤ 2.5 Hz). The LF band is affected by the actions of both the sympathetic nervous system and parasympathetic nervous system, whereas the HF band is related to the parasympathetic control and to respiratory oscillations, either mediated by the vagus nerve or by the direct mechanical action of respiration on the pericardium (1-4, 26, 27, 30, 36, 37, 41).

The baseline value of BRS was assessed on a 3 minute window at the beginning of the protocol, before SAO. The BRS values were then tracked during the observation period from 30’ after SAO until FPD, by considering 3 minute long, 50% overlapped windows. Three of these windows in the first 6 minutes of this period and three in the last 6 minutes were averaged to obtain the values relevant to the periods named respectively “30’ after SAO” and “before FPD”.

F. Statistical analysis

The comparisons among the four groups (A, B, C, D, see Table I) were performed by means of one-way ANOVA. For the first period, i.e. from baseline until 30’ after the initiation of SAO, the comparisons of the values at four time points (i.e. baseline, maximum value and minimum value of mean arterial pressure, 30’ after SAO) were performed with repeated measurements one-way ANOVA for each experimental groups. The Tukey-Kramer test was applied for the post-hoc comparisons.
For the second period, i.e. from “30’ after SAO” until FPD, paired Student’s t-tests were utilized to compare the values of each variable of interest from the two groups SFPD and FFPD at two time points, that is at 30’ after SAO and immediately before FPD. The comparisons between the SFPD and FFPD groups were performed by a Student’s t-test.

III. RESULTS

A. Time domain analysis

The effects of artery ligation and SAO (Figures 1, 2) consisted in a sudden spike in ABP after clamping, and acceleration of the heart beat, followed by a rapid rebound towards lower values. Before SAO (baseline), the animals treated with Xylazine showed significantly lower MAP and higher HP, i.e. were in a state of hypotension (Table II) and bradycardia (Table III, Figure 2). The immediate effect of the ligation of the arteries feeding the splanchnic circulation resulted in a sizable spike of arterial pressure (Figure 1, Table II) and a moderate increase in heart rate (Figure 2, Table III), calculated as the inverse of heart period, until a maximum value was reached. Interestingly, the maximum value of MAP was not different among the four groups. Heart rate was significantly larger after artery ligation only in the rats treated with Xylazine (C and D groups).

In the window starting from 30’ after SAO and lasting until FPD, the comparison of FFPD vs. SFPD animals (Table IV) showed that MAP decreased progressively in both groups, but less markedly in FFPD than in SFPD. The heart period became longer in FFPD and shorter in SFPD. An important feature of the FFPD animals is evident upon simple visual inspection of the mean arterial pressure tracing during the approach to the fatal drop. Before collapse, there are several dips of variable entity (Figure 3) that are promptly compensated, although the pressure value before the dip is not fully recovered. In the following, we will refer to these episodes as *dip and recovery episodes*. Thus, the collapse that leads to the loss of the pulse and therefore to death appears to be preceded by these pressure dips.
B. Baroreflex sensitivity

The BRS gain in the LF band significantly increased in FFPD during intestinal ischemia, whereas significantly decreased in SFPD group (Table IV). The range of the estimates of BRS gain is in agreement with previous works (39, 43). No significant differences were found in the HF band.

C. Heart Period Characteristics (HPC) and Blood Pressure Characteristics (BPC).

The computation of the sample asymmetry was performed on 3 minute long, 50% overlapped running windows across the entire period between 30’ after SAO and the end of the arterial pressure record. The increasing value of the R1/R2 index was able to track the sudden variations in arterial pressure (Figure 3) in correspondence with the dip and recovery episodes. The sample asymmetry of the distributions of heart period and mean arterial pressure (Figure 4) was also computed in the whole time window between 30’ after SAO and FPD, as explained in the Methods section. The R1/R2 ratio highlighted the prevalence of episodes of deceleration of the heartbeat in FFPD rats, whereas a prevalence of accelerations was found in SFPD rats (FFPD R1/R2: 0.76 ± 0.57 vs. SFPD R1/R2: 1.94 ± 1.27, p-value = 0.04). For the mean arterial pressure series, a higher prevalence of episodes of pressure drops in FFPD rats was obtained with respect to SFPD rats (FFPD R1/R2: 3.02 ± 2.87 vs. SFPD R1/R2: 1.47 ± 1.29 p-value = 0.08).

IV. DISCUSSION

In order to track the status of the autonomic control of circulation during SAO, and propose a quantitative characterization of the risk for sudden cardiovascular death, we investigated: a) the features of the responses to the induction of SAO; b) the evolution towards collapse, that is the different behavior of FFPD vs. SFPD animals, during the period of time in which a steady pressure drop occurs, after the response to the onset of SAO by artery ligation vanished. Thus, the discussion is organized accordingly, and divided into two main subsections.
A. Effect of SAO induction

The reduced baseline values of mean arterial pressure and heart rate (indicated by a longer heart period) in the groups C and D (Table II, III) were consistent with the sympatholytic and tranquilizing action of xylazine (14, 19, 35). The steep raise in arterial pressure to similar values in all four groups was independent of the pre-ligation treatment and baseline values. Arterial pressure was remarkably more sensitive to the maneuver than heart period, which changed significantly from baseline only in the rats that received xylazine. For these reasons, it can be observed that the spike in ABP was largely caused by the sizable increase of resistance, of mechanical origin (ligation of the arteries and interruption of blood flow to an important vascular district such as the mesenteric circulation), possibly modulated by a sympathetic mediated vasoconstriction.

Then, we sought to isolate the short-term effects of SAO, and we deemed necessary to limit the window of observation to the first 30’ after the ligation, and to study the long-term effects of shock from that time point on. We opted to analyze a period of such duration based on previous evidence of the degree of intestinal damage that ischemia induces over such time intervals, as previously observed in Schmid-Schönbein’s laboratory. Besides, the trends of the majority of the groups at 30’ after SAO pointed to a recovery of the baseline values of heart rate, and to the fact that a progressive decline of arterial pressure was under way.

Nevertheless, the posthoc comparisons following one-way ANOVA to evaluate the differences between the four treatments at 30’ after SAO showed another important result. In the case of mean arterial pressure, the xylazine + glucose group (C) was different from the non-xylazine groups (A, B); in the case of heart period, both xylazine groups (C, D) were different from the A and B groups. These results showed a trend towards the recovery of pre-SAO values from the standpoint of the differences between xylazine treated and untreated rats, and could be interpreted as an indication that the mechanical effects of SAO induction are compensated during the first 30’ after SAO.

The proposed approach of limiting the compensation to SAO to a well-defined time window for all the experimental groups, independent both of the initial treatments and of the final outcome,
addressed one of the questions which were left unanswered in our previous study (31). In it, it was observed that the trend of ABP reduction towards the fatal drop progressed through an “initial drop”, following the “initial rise” provoked by artery ligation, and preceding the final fatal drop. However, the features and duration of the “initial drop” were not quantitatively described. In short, the analysis of the consequences of SAO induction showed that the pharmacological and fluid treatments did not cause different adaptations to the early stage of ischemia.

B. Progression towards the fatal pressure drop: Baroreflex Sensitivity

The second objective was to gain insight into the impairment of physiological control of circulation in shock due to prolonged intestinal ischemia, by assessing baroreflex sensitivity in the FFPD vs. SFPD groups. No significant differences were found in the HF band between the FFPD and SFPD groups, and for this reason only the results in the LF band are shown (Table IV).

Mean arterial pressure was significantly lower both in FFPD and SFPD rats before the FPD with respect to 30’ after SAO; however, there was no significant difference between the two groups at the two time points. In terms of heart period, the two groups displayed an opposite trend: in the FFPD rats, the duration of the heart cycle was longer (although not significantly) before the FPD than at 30’ after SAO, while it was shorter (significantly) for the SFPD rats. The difference (∆) between the values at FPD and at 30’ after SAO were significantly different for the SFPD and FFPD rats, showing that the heart rhythm was slower in the FFPD rats. This result suggested the existence of a compensating mechanism causing an elevated heart rate in response to the decreasing arterial pressure in the animals that were able to prevent the collapse.

The LF gain of the baroreflex was significantly increased at FPD with respect to 30’ after SAO in the FFPD group and decreased in the SFPD group. Besides, the values for the SFPD and FFPD groups were different before FPD. These results are particularly interesting because they hint at a higher responsiveness of the cardiac baroreflex in FFPD rats. Therefore, the joint observation of the time domain analysis of heart period and of the BRS at LF for the FFPD rats showed that the
cardiac baroreflex is enhanced during ischemia (possibly for a sympathetic mechanism, given that no difference was found in the vagal band), but this does not translate into an actual acceleration of the heart beat. Thus, it could be hypothesized that, besides a vagal mechanism, the occurrence of a sudden collapse could also depend on the impairment of the sympathetic control, which is not only unable to maintain blood pressure, but also effectively regulate heart rate as it should be expected in the presence of an enhanced BRS.

Such a role of the sympathetic system is also indicated by the observation (Figure 3) of the presence of the “dip and recovery” episodes. Indeed, a prompt response to compensate a sudden drop in pressure, even if only in part, can be ascribed only to a baroreflex response on vessels that is mediated by the sympathetic nervous system. This may lead to the question whether the sympathetic system was actually overactivated in FFPD rats, as a response to prolonged gut ischemia. However, such a scenario would be consistent with the reported ability of gut ischemia to enhance sympathetic activation (24). This overactivation could be so sustained that it enabled to compensate for several dips in arterial pressure, even of large entity (Figure 3). A saturation of the sympathetic mediated baroreflex responsiveness could have been reached upon the repeated challenge represented by the numerous dip and recovery episodes, though, and this would not only explain the arterial pressure fall that led to collapse, but also the inability of the system to increase heart rate before collapse.

In the end, if the hypotheses of sympathetic overactivity and saturation or inhibition are valid, when the sympathetic system becomes unable to react to a protracted stimulation, such as ischemia without reperfusion, a purely “sympatho-vagal balance” like interpretation would justify a sudden shift to a predominantly vagal mediated control, that exacerbates the inability of the sympathetic system to maintain the vascular tone by producing a marked decrease in heart rate. The combination of these two phenomena may ultimately cause sudden pulse loss and death. It should not be discarded, though, that one of the reasons why the HF gain of the baroreflex did not display any
significant variation from 30’ after SAO to pre FPD or difference between SFPD and FFPD could rely on the deceleration of the breathing frequency under anesthesia, during shock.

C. Progression towards the fatal pressure drop: Heart Period Characteristics and Blood Pressure Characteristics

The final objective of our work was the computation of indices with the potential to predict the catastrophic outcome of sudden cardiovascular collapse, and for the discrimination of FFPD and SFPD animals. In this regard, both the “Heart Period Characteristics” and the “Blood Pressure Characteristics” shed further light on the differences between FFPD and SFPD rats, and discriminated opposite trends in the adaptation of HP and ABP to prolonged intestinal ischemia. As regards HPC, the results went in the direction of supporting the vagal hypothesis for sudden collapse, since a value of R1/R2 lesser than the unity indicates the prevalence of reductions of heart rate. The opposite was found with the SFPD group, which could have maintained a more physiologic status of the sympatho-vagal balance, particularly in terms of the ANS responsiveness to the challenge represented by ischemia. As to BPC, the value of R1/R2 in the FFPD group was sensibly larger than 1 and larger than the value of SFPD. This could also support the previously formulated hypothesis of a sympathetic overactivity in the FFPD rats, which is elicited by the need for compensating the repeated dips in arterial pressure that cause hemodynamic instability.

Limitations

One of the limitations of this work is the lack of direct measurements of sympathetic and/or vagal activity to corroborate the hypotheses of the overactivity of the sympathetic system and sudden shift in the sympatho-vagal balance towards a vagal predominance in the FFPD rats before collapse. Future protocols with intestinal ischemia need to include measurements of sympathetic discharge,
for instance, in order to verify if an abnormally high sympathetic discharge is associated with a higher risk of hemodynamic instability and cardiovascular collapse and death.

V. CONCLUSION

Despite the large body of work on SAO as an experimental model to induce circulatory shock, to our knowledge the problem of determining the status of autonomic nervous system control of circulation and the features of arterial pressure had not been addressed previously. The results presented in this paper showed that:

i) SAO induction has essentially a mechanical effect on ABP and little effect on heart period, and the pre-treatment with drugs interfering with the sympathetic and parasympathetic systems does not change the response to the ligation of the arteries that feed the mesenteric circulation;

ii) After the mechanical perturbation of SAO vanished, autonomic control adapted to ischemia and had to cope with a steady pressure drop. The most catastrophic scenario is sudden cardiovascular collapse, unless the vagal system is not properly inhibited (31). In this case, the increased gain in the LF band of the baroreflex control of heart rate hinted at the presence of sympathetic overactivity. The latter interpretation is further supported by the observation that the tracing of arterial pressure in FFPD rats was affected by numerous hypotensive episodes, that were partially compensated until the system was unable to respond, causing a loss of the pulse. Hence, if the vagal system is not blocked, it can prevail in the autonomic regulation of circulation, and cause the paradox effect of contributing to slowing down the heart until stopping the beat, by combination of the reduction of heart rate (direct action of the parasympathetic system on the sinus node), and a lack of arterial pressure maintenance (failure of sympathetic control of vasomotor tone).

iii) The Sample Asymmetry parameter derived from the two functions Heart Period Characteristics and Blood Pressure Characteristics was able to discriminate between FFPD and SFPD rats. These indices could have predictive value and a further confirmation in animal studies could support their
use in blood pressure records from critically ill patients, and for a potential translational use in bedside applications. In fact, our results further support Moorman and colleagues’ reports on the diagnostic importance of Heart Rate Characteristics. In particular, Heart Period Characteristics was able to significantly discriminate FFPD an SFPD rats (p-value < 0.05), whereas the Blood Pressure Characteristics comparison was only marginally significant (p-value=0.08). Still, this trend justifies the use of this approach to larger populations, especially of arterial pressure recordings from critically ill patients, in order to verify the potential clinical usefulness of Heart Rate/Period Characteristics and Blood Pressure Characteristics as predictors of sudden death.

An important question that remains open is whether the absence of baseline data (i.e., “healthy blood pressure records”) somehow affects the validity of the proposed approach. In fact, in highly uncontrolled conditions such as an intensive care unit, the absence of observations on the autonomic status and the characteristics of blood pressure and ECG before shock could hamper the robustness of the calculated indices, besides the well-known factor of inter-subject variability. Still, the results that have been presented in the manuscript show the validity of these methods in animal studies, and support the need for their application in real patients records.

Although the patho-physiological interpretation of the results obtained by the baroreflex analysis, and by the application of the two SA based indices named Heart Period Characteristics and Blood Pressure Characteristics could be affected by limitations implicit in the black box approach underlying their use, the improvement of currently available algorithms could benefit the introduction of new technologies in the critical care setting. The results presented in this manuscript suggest the usefulness of ever more robust combinations of data analysis approaches, where standard physiological models (e.g., BRS) and “data mining” like approaches, that are blind to the fundamental mechanisms (e.g., nonlinear analysis of heart rate and blood pressure) mesh and enhance the reliability of patient risk prediction.
Acknowledgments

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**Table I.** Experimental groups

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th># rats</th>
<th>#FFPD</th>
<th>#SFPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: NoXyl Glucose</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>B: Only Saline</td>
<td>7</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>C: Xyl Glucose</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>D: Xyl Gly Glucose</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>
Table II. Mean arterial pressure (mmHg) values for each group and each phase before and after ligation of the mesenteric and celiac arteries (before SAO, maximum value after SAO, minimum value and value at the end of the 30 minutes after clamping).

<table>
<thead>
<tr>
<th></th>
<th>Before SAO*</th>
<th>Maximum Value</th>
<th>Minimum Value</th>
<th>30' after SAO*</th>
<th>One-way ANOVA for repeated measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: NoXyl Glucose</td>
<td>111 ± 9</td>
<td>145 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>121 ± 23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>134 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>B: Only Saline</td>
<td>122 ± 4</td>
<td>145 ± 9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>126 ± 6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>129 ± 7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>C: Xyl Glucose</td>
<td>76 ± 15&lt;sup&gt;a&lt;/sup&gt;,&lt;sup&gt;§&lt;/sup&gt;</td>
<td>145 ± 9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>106 ± 12&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>107 ± 13&lt;sup&gt;a,b&lt;/sup&gt;,&lt;sup&gt;§&lt;/sup&gt;</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>D: Xyl Gly Glucose</td>
<td>79 ± 14&lt;sup&gt;a&lt;/sup&gt;,&lt;sup&gt;§&lt;/sup&gt;</td>
<td>155 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>117 ± 15&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>120 ± 10&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

Comparisons among different groups at each time point: *one-way ANOVA p-value < 0.01, post hoc comparisons p-value < 0.05 vs. group A, post hoc comparisons p-value < 0.05 vs. group B.

Comparisons among different time points: post hoc comparisons p-value < 0.05 vs. before SAO, post hoc comparison p-value < 0.05 vs. maximum value.
Table III. Heart Period values (msec) for each group and each phase before and after ligation of the mesenteric and celiac arteries (before SAO, minimum value after SAO, maximum value and value at the end of the 30 minutes after clamping).

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>Minimum value*</th>
<th>Maximum value*</th>
<th>30' after SAO*</th>
<th>One-way ANOVA for repeated measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A: NoXyl Glucose</strong></td>
<td>176 ± 15</td>
<td>166 ± 10</td>
<td>190 ± 21(^b)</td>
<td>186 ± 16</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td><strong>B: Only Saline</strong></td>
<td>171 ± 29</td>
<td>163 ± 24</td>
<td>187 ± 28(^a)</td>
<td>186 ± 27(^b)</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td><strong>C: Xyl Glucose</strong></td>
<td>287 ± 30(^a),(^$)</td>
<td>239 ± 20(^a),(^l),(^a)</td>
<td>287 ± 21(^a),(^l),(^b)</td>
<td>276 ± 23(^a),(^l),(^b)</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td><strong>D: Xyl Gly Glucose</strong></td>
<td>249 ± 22(^a),(^$)</td>
<td>204 ± 28(^l),(^a)</td>
<td>270 ± 20(^a),(^l),(^b)</td>
<td>263 ± 30(^a),(^l),(^b)</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

Comparisons among different groups at each time point: *one-way ANOVA p-value < 0.01, \(^a\)post hoc comparisons p-value < 0.05 vs. group A, \(^b\)post hoc comparisons p-value < 0.05 vs. group B.
Comparisons among different time points: \(^a\)post hoc comparisons p-value < 0.05 vs. before SAO, \(^b\)post hoc comparisons p-value < 0.05 vs. minimum value.
Table IV. Average values of mean arterial pressure (MAP, mmHg), heart period (heart period, msec) and baroreflex gain (BRS, ms/mmHg) estimated in LF band (BRS_{LF}) for each group and for each phase considered (30 minutes after SAO and before FPD episode) during the progression towards the fatal pressure drop.

<table>
<thead>
<tr>
<th></th>
<th>30’ after SAO</th>
<th>Pre FPD</th>
<th>Δ=PreFPD - 30’ after SAO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FFPD MAP (mmHg)</strong></td>
<td>123 ± 12</td>
<td>105 ± 23(^b)</td>
<td>-19 ± 15</td>
</tr>
<tr>
<td><strong>SFPD MAP (mmHg)</strong></td>
<td>118 ± 17</td>
<td>91 ± 17(^a)</td>
<td>-27 ± 9</td>
</tr>
<tr>
<td><strong>FFPD HP (msec)</strong></td>
<td>220 ± 47</td>
<td>238 ± 58</td>
<td>18 ± 27(^*)</td>
</tr>
<tr>
<td><strong>SFPD HP (msec)</strong></td>
<td>230 ± 54</td>
<td>211 ± 50(^a)</td>
<td>-19 ± 27(^*)</td>
</tr>
<tr>
<td><strong>FFPD BRS(_{LF}) (ms/mmHg)</strong></td>
<td>0.22 ± 0.11</td>
<td>0.36 ± 0.15(^**)</td>
<td>0.142 ± 0.072(^*)</td>
</tr>
<tr>
<td><strong>SFPD BRS(_{LF}) (ms/mmHg)</strong></td>
<td>0.25 ± 0.21</td>
<td>0.19 ± 0.12(^**)</td>
<td>-0.053 ± 0.157(^*)</td>
</tr>
</tbody>
</table>

Comparison between phases: paired t-test (Pre FPD vs. 30’ after SAO), \(^a\)p-value < 0.01, \(^b\)p-value < 0.05. Comparisons FFPD vs. SFPD: *unpaired t-test p-value < 0.01.
Figure 1. Example of mean arterial pressure tracing during an experiment ending with a fast fatal pressure drop and sudden cardiovascular death.
Figure 2. Evolution of heart rate for the four experimental groups, from the baseline level preceding clamping of splanchnic arteries (preCL), to the maximum and minimum values achieved following clamping, and until the final value (end) in the 30’ window chosen for analysis following ligation of the arteries and SAO initiation.
Figure 3. One-hour long window of mean arterial pressure (dark) during the steady decrease towards fatal pressure drop, and its sample asymmetry (light) tracked by calculations over successive, 3 minute long, 50% overlapped running windows. The *dip and recovery episodes* were identified by sudden increases in sample asymmetry.
Figure 4. Sample asymmetry distributions of heart period (Heart Period Characteristics, HPC) and mean arterial pressure (Blood Pressure Characteristics, BPC), FFPD vs. SFPD groups.
REFERENCES


