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Using the Interrelationships between Rat Arterial Pulse Waveform Parameters to Study Connections/Disconnections between NO Signaling Pathways

Karol Ondrias

Abstract

Background: Nitric oxide **(**NO) signaling pathways have been thoroughly investigated at molecular level, but connections/disconnections among NO pathways are not yet fully understood.

Objective: The aim of the study was to investigate which of the 44 parameters of the rat arterial pulse waveform (APW-Ps) are regulated by the same or different signaling pathways as systolic blood pressure (Sys.BP).

Methods: The right jugular vein of anesthetized spontaneously hypertensive (SHR) rats was cannulated for i.v. administration of NO donor S-nitrosoglutathione (GSNO). The left carotid artery was cannulated to detect and calculated APW-Ps. The interrelationships between APW-Ps in hysteresis and non-hysteresis modes in SHR rats was evaluated to suggest connection between different signaling pathways.

Results: At least 13 of 43 hemodynamic APW-Ps, which are differently regulated by NO than is regulated Sys.BP was suggested. These include heart rate, dP/dt_{max}, interval between anacrotic notch and diastolic BP or dicrotic notch relative delay. Comparison GSNO effect in SHR and normotensive rats revealed that their interrelationships are significantly different and even produce opposite results. Di-gital, tri-gital, and tetra-gital temporal movement of Sys.BP on the APW was observed during the effects of GSNO.

Conclusion: It is suggested that hysteresis and non-hysteresis modes of interrelationships between APW-Ps are suitable for investigating connection/disconnection between different signaling pathways. Using this approach, several NO signaling pathways were suggested. From a clinical perspective, this approach may be used in animal models and in humans to create a data bank of APW-Ps and their interrelationships patterns for different cardiovascular conditions.

Keywords: Arterial pulse waveform; Blood pressure; Hemodynamic parameters; Signaling pathways; Nitric oxide; SHR rat

Introduction

Nitric oxide (NO) is involved in multiple physiological processes. It is critical signaling molecule in the cardiovascular system, playing a fundamental role in the regulation of vascular tone, blood flow, and blood pressure (BP). Its diminished bioavailability contributes to endothelial dysfunction, vascular stiffening, and impaired cardiac performance [1-4]. NO signaling pathways have been thoroughly investigated at molecular level [3, 4], but connections/disconnections among NO pathways are not yet fully understood. It is

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hypothesized that the shape of the arterial pulse waveform (APW) may provide information about the cardiovascular system or pathological conditions of the body [5-10]. To confirm this hypothesis, it is necessary to characterize the APW under various physiological conditions and associate 'APW pattern' of the condition with a specific state of the organism. Therefore, the time-dependent measurement and evaluation of 35 or 57 APW parameters (APW-Ps), from which some are established hemodynamic parameters, have been introduced [5, 11]. Using the time-dependent data of transient effects of some compounds on APW, it was possible to study interrelationships between APW-Ps [12-14]. It has been observed that some interrelationships showed hysteresis, but other not.

In previous study with xylazine/Zoletil (Xy/Zo) anesthetized normotensive (NORM) rats, the effect of the NO donor, S-nitrosoglutathione (GSNO), on the interrelationships between several APW-Ps has been investigated [12]. The interrelationships with or without hysteresis have been observed between systolic blood pressure (Sys.BP) and other APW-Ps. It has been suggested that interrelationships without hysteresis between two APW-Ps indicate that their signaling pathways, responsive to NO, are directly interconnected. On the other hand, interrelationships with hysteresis suggest that these two signaling pathways are connected indirectly or not at all. It has been hypothesized that from studies of interrelationships between different APW-Ps it is possible to determine how the two signaling pathways are connected [12]. The aim of our study was to investigate interrelationships between hemodynamic APW-Ps in spontaneously hypertensive (SHR) rats, in order to suggest connections/disconnections between NO signaling pathways. Therefore, I investigated the time-dependent effects of GSNO on 44 APW-P in SHR rats and the interrelationships between 43 APW-Ps and Sys.BP to determine whether and how 43 APW-Ps signaling pathways are linked to the Sys.BP pathway. This study presents only interrelationships between the 43 APW-Ps and Sys.BP, which account for just 4.5% of the total 946 interrelationships among pairs of the 44 APW-Ps.

I wanted also determine whether the effect of GSNO on APW-Ps in SHR rats is generally applicable to rats under other physiological conditions as well. Therefore, I compared the effect of GSNO on SHR rats under isoflurane (ISO) anesthesia with its effect on NORM rats under Xy/Zo anesthesia. Data of GSNO effects on APW-Ps and hysteresis/non-hysteresis interrelationships obtained from SHR rats were compared with the published data of NORM rats [12].

Material and Methods

S-Nitrosoglutathione (GSNO; N4148) was purchased from Sigma-Aldrich (Schnelldorf, Germany) and Isoflurin (Isoflurane, 1,000 mg/g) from Vetpharma (Barcelona, Spain). In the case of SHR rats, new data of APW were obtained from original records published in [17] and evaluated from three more SHR rats (adult male SHR rats; 280–320 g; 17–19 weeks old). Rats were obtained, received veterinary care [15], anesthetized with isoflurane (ISO), underwent surgery, recording of APW and evaluation of APW-Ps were performed as described in our previous studies [11, 16, 17]. The right jugular vein of ISO anesthetized rats was cannulated for intravenous (i.v.) bolus administration of GSNO. The left carotid artery was cannulated for the detection of APW from which 44 APW parameters were evaluated using a microcatheter pressure transducer [12]. For details of method, definition and abbreviation of 44 APW-Ps calculated from ten points **a - j** on APW are provided in Supplementary information Figure S1 [5, 11]. In the case of NORM rats, the data were obtained from original recording published in [12].

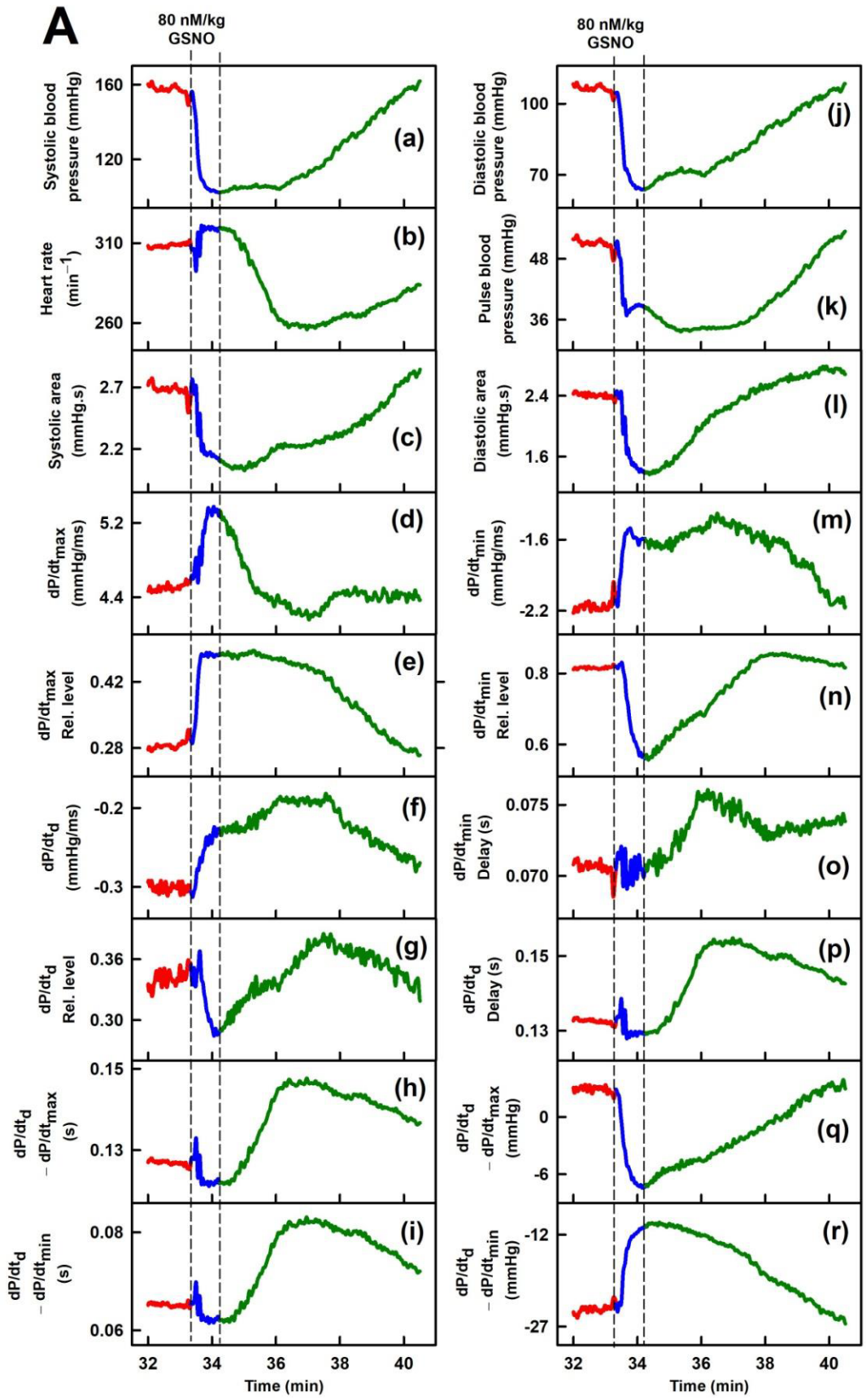
Results

The time-dependent effects of NO-donor GSNO on 44 APW-Ps of SHR rats

To characterize the time-dependent effect of GSNO, APW-Ps were evaluated from APW of SHR rats in seven experiments, labeled Exp1–Exp7 (Supplementary information Figures S2,S3A,B,C - S9A,B,C). Administration of GSNO transiently influenced all APW-Ps (Figure 1). It is interesting to note that every time-dependent APW-P exhibited a characteristic 'APW pattern'. GSNO transiently decreased SyS.BP and changed other APW-Ps. Qualitatively evaluated transient changes of 44 APW-Ps in seven experiments are shown in Supplementary information Table S1. Since the time-dependent maximum effect of GSNO differed for different APW-Ps, I compared the time shift of the maximum effect of GSNO of APW-Ps to the maximum effect of Sys. BP in SHR rats (Table S1 - Time shift). I can suggest that the shift of the maximum effect towards higher (or lower) time values indicates that the signal pathway characterized by the given APW-P is slower than the signal pathway for Sys.BP (and vice versa).

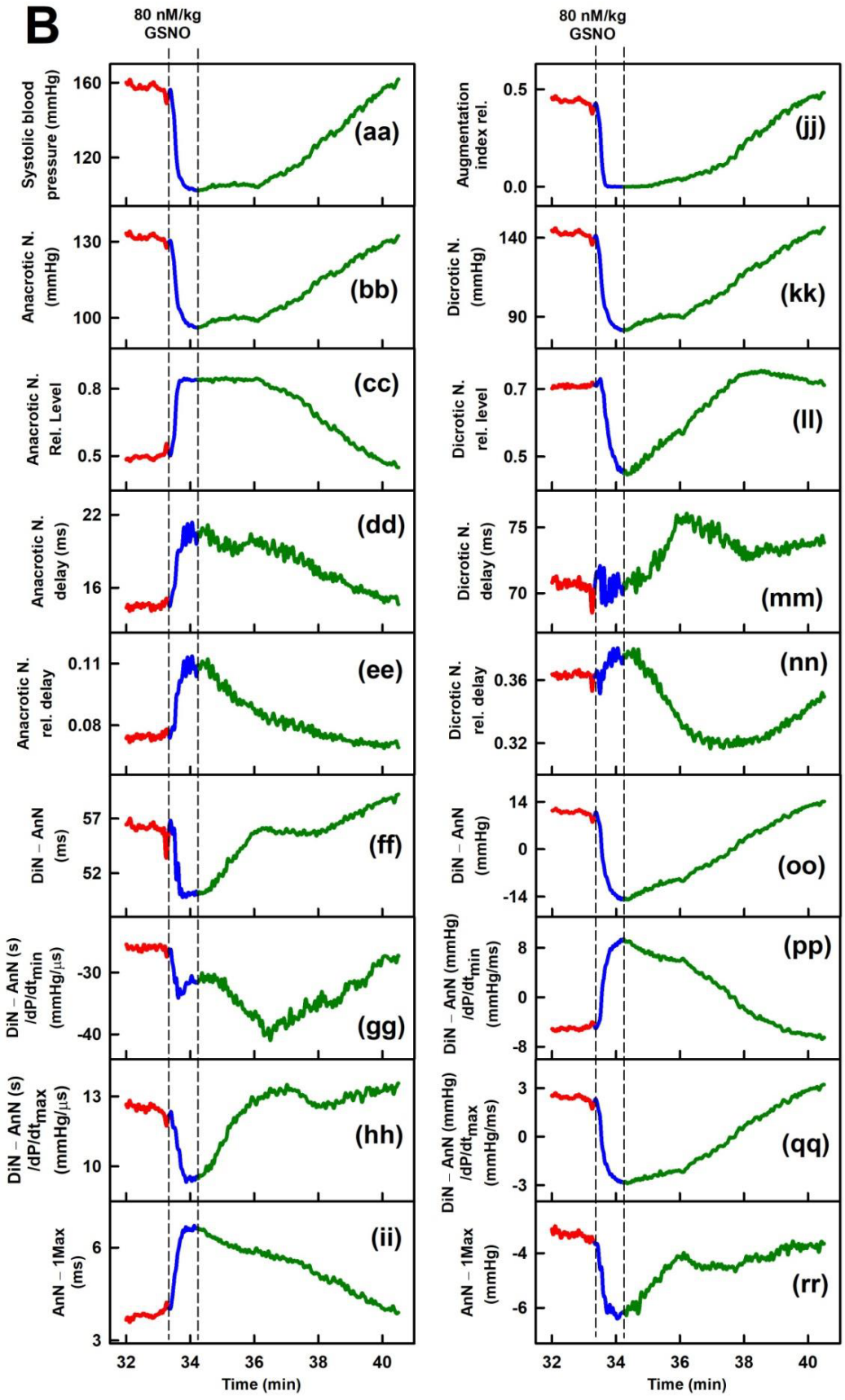
In a previous study, the effect of i.v. administration of GSNO on NORM rats under Xy/Zo anesthesia has been investigated [12]. In these experiments, 32 nmol/kg GSNO was used, since NORM rats were more sensitive to GSNO than SHR rats. Therefore i.v. administration of 80 nmol/kg GSNO was used in SHR rats. A comparison of recent APW-Ps data for SHR rats (Table S1) with published 35 APW-Ps data for NORM rats (Table 1 in [12]) shows that in both animal models, increase or decrease APW-P was similar in APW-Ps: (a), (d), (j), (l), (q), (r), (bb), (cc), (dd), (ee), (hh), (ii), (kk), (oo), (pp) and (qq). For the other parameters, the comparison effect of GSNO on APW-Ps was not clearly recognized (Table S1). Notably, some APW-Ps has transient,

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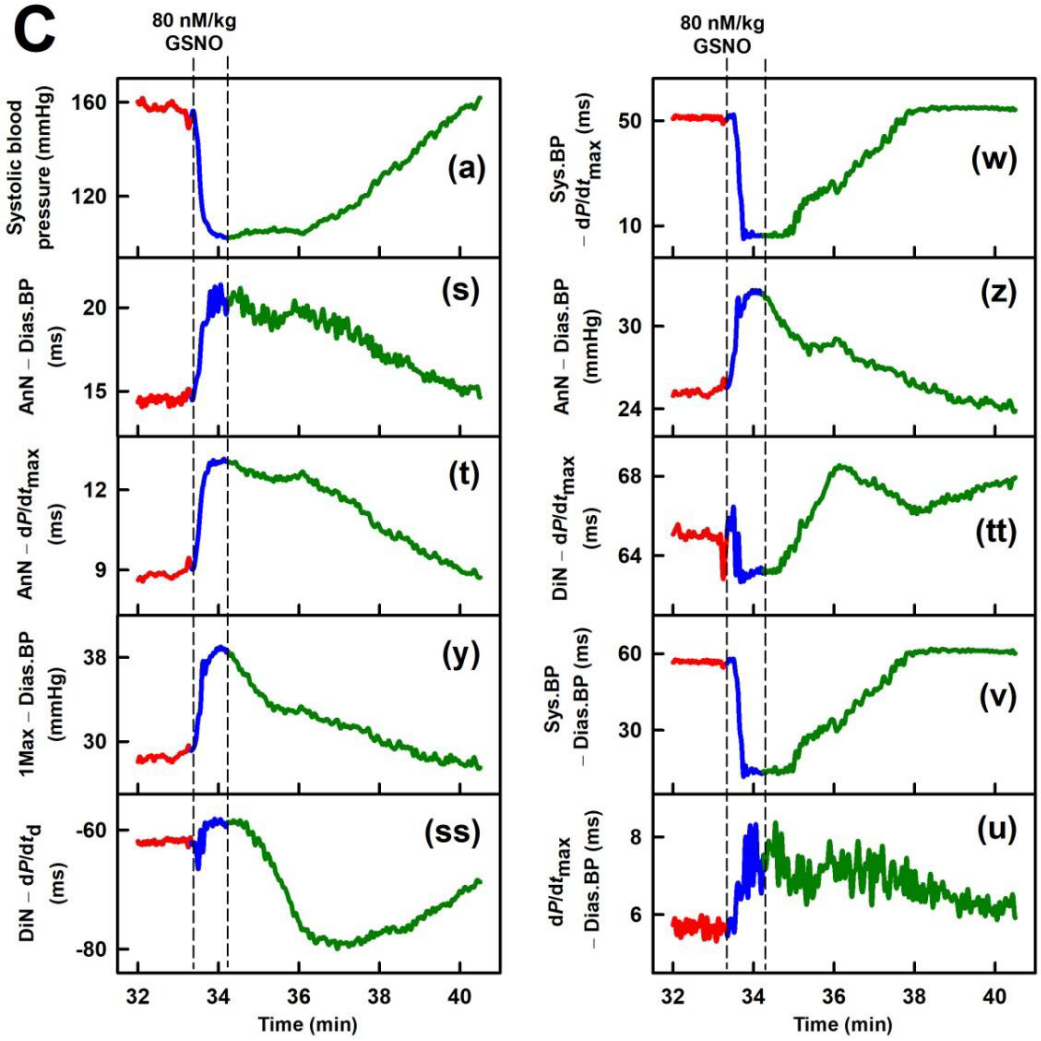


Figure 1A, B, C: Exp-1. Time-dependent changes in 44 hemodynamic APW-Ps of anesthetized SHR rats after bolus administration of 80 nmol/kg GSNO (left dashed line). Control (red), decrease of Sys.BP after GSNO administration (blue) and return the Sys.BP to control (green). The right dashed line marked minimum of Sys.BP after GSNO administration. Definitions, units and abbreviations of APW-Ps evaluated from the APW are as explained previously [5, 11] and briefly in Supplementary material Figure S1

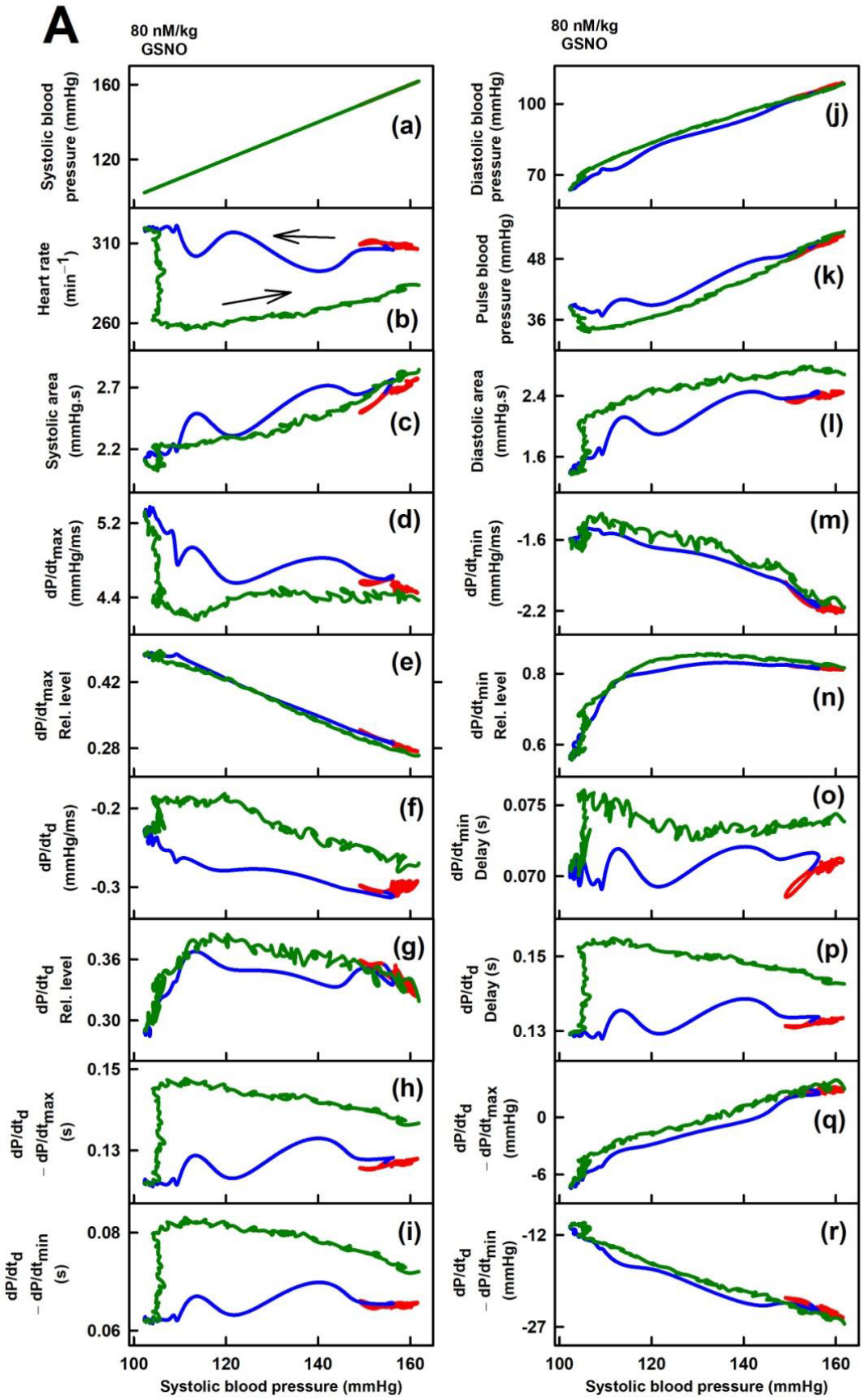
but opposite time dependent response to GSNO in NORM and SHR Rats, as e.g. (c), (e), (k), (m) and (gg) (Figure S10).

Interrelationships of hemodynamic APW-Ps with Sys.BP after administration of GSNO

In our previous studies, it was observed that some APW-Ps interrelationships exhibit hysteresis, which may indicate different signaling pathways for different pairs of APW parameters [12-14]. To investigate whether GSNO induces hysteretic/non-hysteretic interrelationships between APW-P in SHR rats, a similar approach and more detailed analysis were used. To suggest, whether NO signaling pathway regulating Sys.BP regulates similarly other 43 hemodynamic APW-Ps, plots of the interrelationships of 43 APW-Ps to Sys. BP was evaluated (Figure 2). When increase and decrease of Sys.BP correlates with the increase and decrease of other APW-P, non-hysteresis is detected. In this case, it was suggested that these two APW-Ps are regulated by the same signaling pathways. It is in the case when blue and green lines have the same dependence on the increase and the decrease systolic BP (Figure 2). Non-hysteresis interrelationships were observed for most of the APW-Ps. However, hysteretic interrelationships were observed between Sys.BP and APW-Ps as it is seen in plots (b), (d), (f), (h), (i), (p), (gg), (hh), (nn), (rr), (y), (z) or (ss) what may indicate that these two APW-Ps, responding to NO concentration, are not directly regulated by the same signaling pathway as Sys.BP.

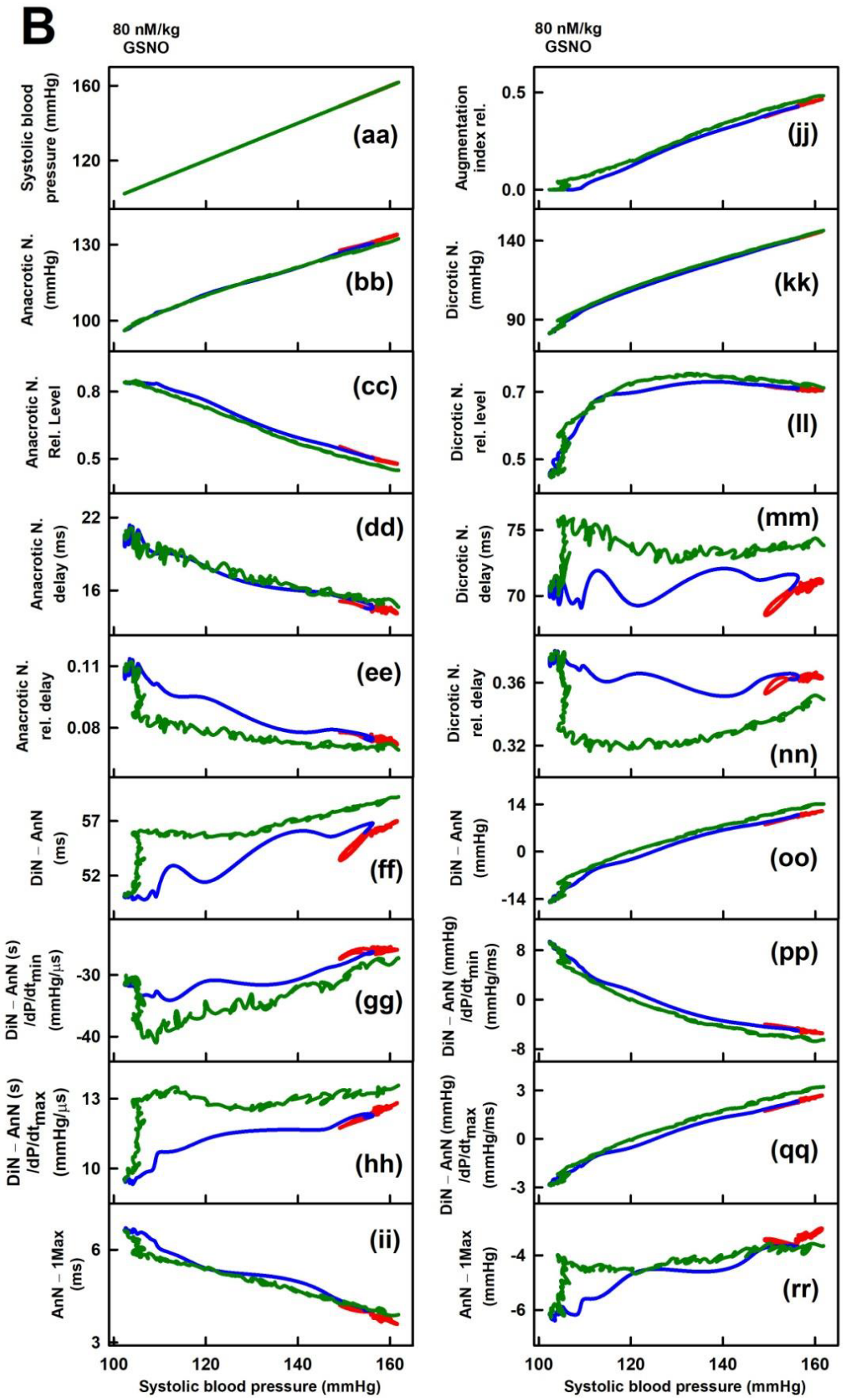
Figure 3 summarizes the number of experiments that represent the non-hysteresis or hysteresis interrelationships of APW-Ps in SHR rats. In at least 5 of 7 experiments, non-hysteretic interrelationships were observed in 25 of 43 interrelationships, and hysteretic interrelationships were observed in 13 APW-Ps. In the remaining 5 APW-Ps, the interrelationships were not clearly established.

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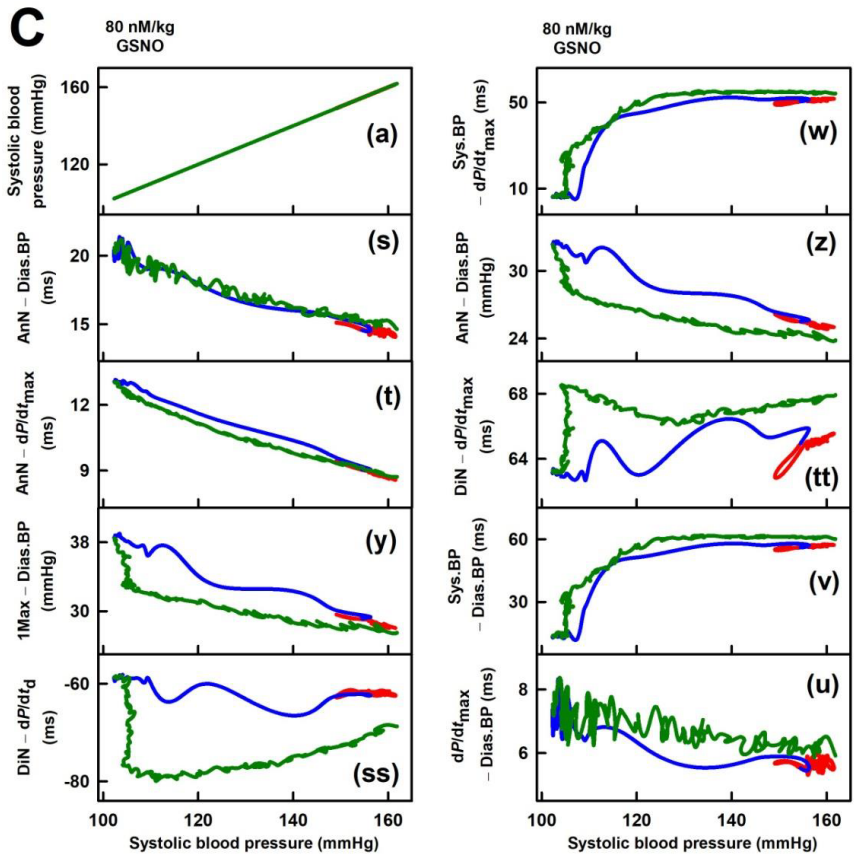


Figure 2A, B, C: Exp-1. Interrelationships of 44 APW-Ps to Sys.BP after bolus administration of 80 nmol/kg GSNO. The data and colors are taken from Figure 1. Red line is control, before GSNO administration. After administration of GSNO, Sys.BP is decreasing (arrow in (b), blue line) and later is increasing to reach control value of Sys.BP (arrow in (b), dark green line)

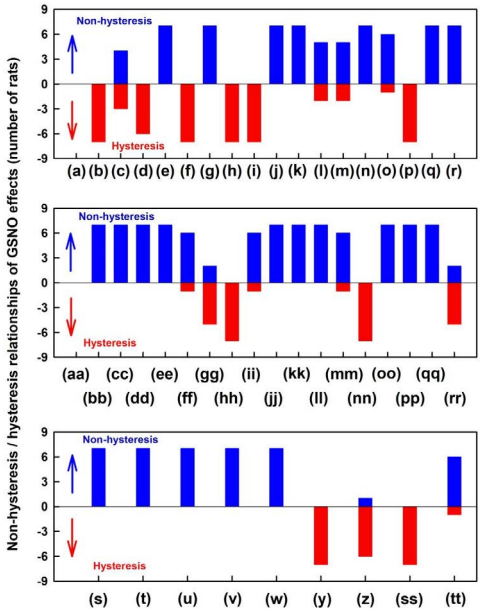


Figure 3: Non-hysteresis/hysteresis interrelationships of hemodynamic APW-Ps to Sys.BP under condition of GSNO administration in SHR rats. The non-hysteresis/hysteresis interrelationships were evaluated from Figure 1 and other 6 experiments, where blue lines represent decrease and green line increase of systolic BP after administration of 80 nmol/kg of GSNO. Number of SHR rats in which non-hysteretics (blue) or hysteretic (red) patterns for the given APW-P were observed (n = 7). Hysteresis was arbitrarily defined as (APW-P – Sys.BP loop) > 40 mmHg of Sys.BP.

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Bellow is the list of the hemodynamic 13 APW-Ps (plots), in which hysteretic interrelationships between Sys.BP and APW-Ps was observed. It suggests NO pathways regulating the hemodynamic APW-Ps, which are not directly connected with the NO pathway regulating Sys.BP in SHR rats. Bold letters bellow represent particular points on APW (Figure S1).

(b) Heart rate in min–1; 60 / (j – a); (j – a) represents time interval between a and j, a and j are two reference points to diastolic BP value on APW.

(d) dP/dtmax in mmHg/ms; maximum derivative at the point b; 'P' is BP in mmHg, 't' is time in ms.

(f) dP/dtd in mmHg/ms; negative derivative at the point i; the point i is the BP in the middle of the time interval between h and j.

(h) dP/dtd – dP/dtmax in s; time interval between points i and b.

(i) dP/dtd – dP/dtmin in s; time interval between points i and g. dP/dtmin is maximum negative derivative at the point g.

(p) dP/dtd delay in s; delay of point i; (i – a) time interval between points i and a.

(gg) [(DiN – AnN) in s] / [dP/dtmin in mmHg/μs] in s/(mmHg/μs); (h – d) / g.

(hh) [(DiN – AnN) in s] / [dP/dtmax in mmHg/μs] in s/(mmHg/μs); Points (h – d) / b.

(nn) Dicrotic notch relative delay; relative delay of point h; (h – a) / (j – a); in ms/ms (dimensionless).

(rr) [AnN in mmHg] – [1Max (point c or the 1st. maximum) in mmHg] in mmHg; (d – c); BP difference between c and d.

(y) [1Max (point c or the 1st. maximum) in mmHg] – Diastolic blood pressure in mmHg; the point a or j. BP interval between 1Max–Dias.BP in mmHg.

(z) [Anacrotic notch in mmHg; BP at the point d] – [Diastolic blood pressure in mmHg; the point a or j]. BP interval between AnN and diastolic BP in mmHg.

(ss) [Dicrotic notch (DiN), point h, in ms] – [dP/dtd; position of negative derivative at the point i, in ms], time interval between h and i in ms, (h – i).

In a previous study, the effect of i.v. administration of GSNO on Xy/Zo anesthetized NORM rats has been investigated [12]. A comparison of APW-Ps interrelationships for SHR rats (Figure 3) with published data for NORM rats (Figure 3C in [12]) shows that in both animal models non-hysteresis was observed in APW-Ps (e), (g), (j), (k), (m), (q), (bb), (dd), (ee), (ii), (kk), (oo) and (pp). In both animal models, hysteresis was observed in APW-Ps (b), (f), (h), (p), (hh) and (rr). For the other parameters, the effect of GSNO on hysteresis/non-hysteresis in these two models differed. Notably, from the comparison of SHR and NORM rats, the interrelationships patterns showed opposite dependences on Sys.BP in APW-Ps (c), (e), (g), (k), (m), (n), (gg) and (ll) (Figure S11). For example, pulse BP (plot (k)) is increasing with increasing Sys.BP in SHR rats, but is decreasing with increasing Sys.BP in NORM rats. Interestingly, details of comparison of the interrelationships patterns in SHR and NORM rats revealed that Sys.BP decrease/increase loop in some APW-Ps had completely opposite trends in these two animal models (Figure 4).

Interrelationships of heart rate (HR) with Sys.BP after administration of GSNO

From 13 APW-Ps which showed hysteretic interrelationships to Sys.BP, I chose to compare in more details the interrelationships of HR to Sys.BP in SHR (Figure S12) and NORM (Figure S13) rats. In SHR rats, GSNO transiently decreased Sys.BP in all seven experiments. HR fluctuated for about 30-50 s and later transiently decreased. The interrelationships of HR to Sys.BP revealed 'open loops' that during decreasing Sys.BP, HR values remained roughly constant and varied only slightly. When Sys.BP decreased more, HR decreased and retained in lower values even after Sys.BP increase (Figure S12). In NORM rats, GSNO transiently decreased Sys.BP in all seven experiments for about 30-50 s, than increased and later may decrease again. The interrelationships of HR to Sys.BP revealed 'open and/or closed loops' that during decreasing Sys.BP, HR values remained roughly constant and varied only slightly. When Sys.BP decreased more, HR increased and retained in values even after Sys.BP increase (Figure S13). In conclusion, it should be noted that in these two animal models the qualitative, but not quantitatively, transient effects of GSNO on Sys.BP were similar; however, the effect on HR and the interrelationships between HR and Sys.BP, were qualitatively opposite.

In SHR rats, the maximal time delayed transient effects of GSNO on Sys.BP and HR were 1 and 2.7 min, respectively. Effect of GSNO on HR was 1.7 min delayed with comparison to effects on Sys.BP. However, in NORM rats, the maximal time delayed effects of GSNO on Sys.BP and HR were 0.45 and 0.16 min, respectively. Effect of GSNO on HR was 0.3 min shorter with comparison to the effects on Sys.BP (Figure 5). In conclusion, it should be noted again that in these two animal models the qualitative, but not quantitative, delayed transient effects of GSNO on Sys.BP were similar; however, the effect on HR and the interrelationships between HR and Sys.BP, were qualitatively opposite.

The n-gital fluctuation of APW-Ps in anesthetized SHR rats after administration of GSNO

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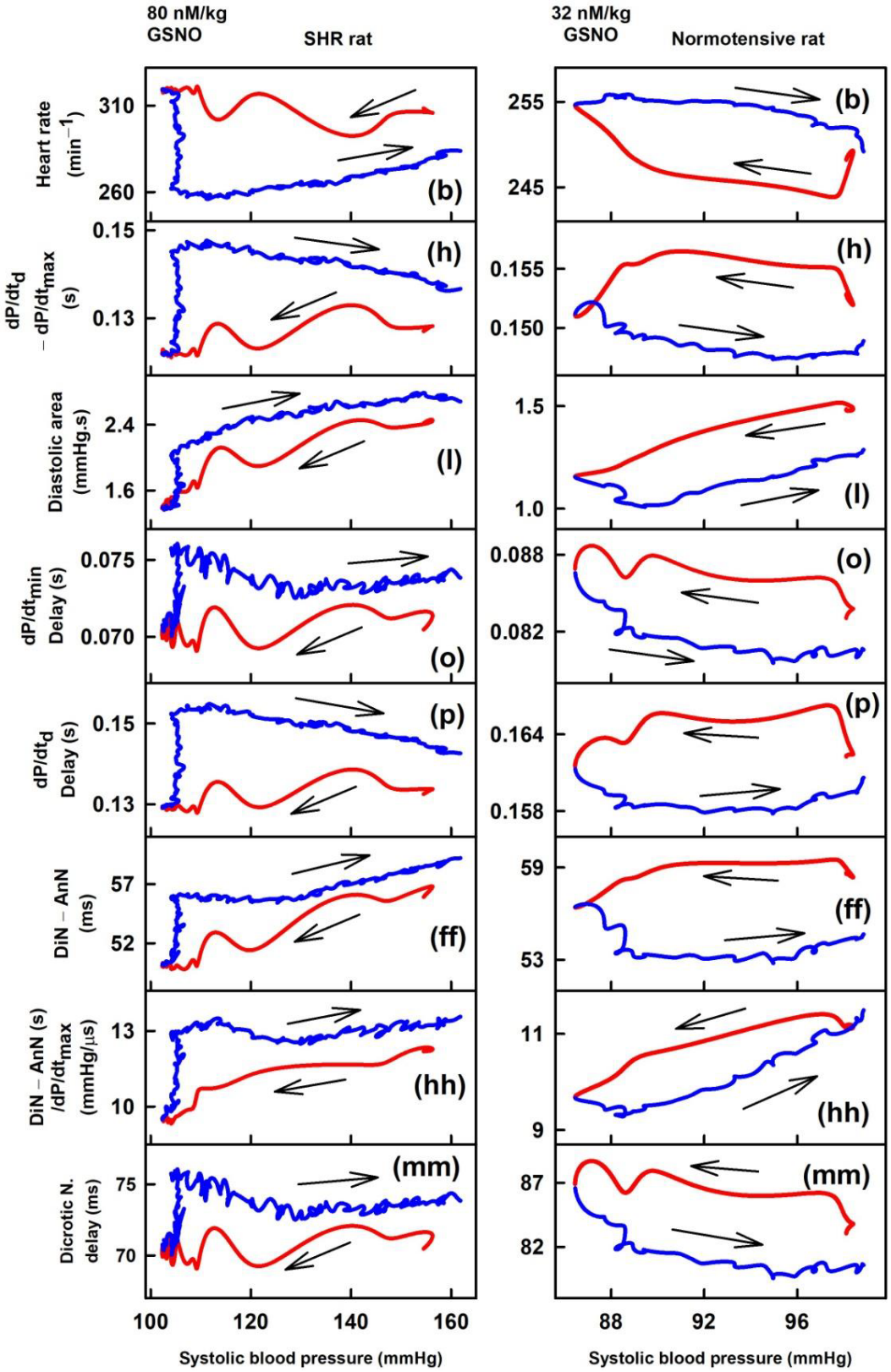


Figure 4: Comparison of detail interrelationships of 8 APW-Ps to Sys.BP in SHR (left plots) and NORM (right plots) rats after bolus administration of 80 nmol/kg and 32 nmol/kg GSNO, respectively. The data for left plots are taken from Figure 2. The data for the right plots are taken from Figure 3B in [12]. After administration of GSNO, Sys.BP is decreasing in both animal models (arrow, red line) and later is increasing to reach control value of Sys.BP (arrow, blue line)

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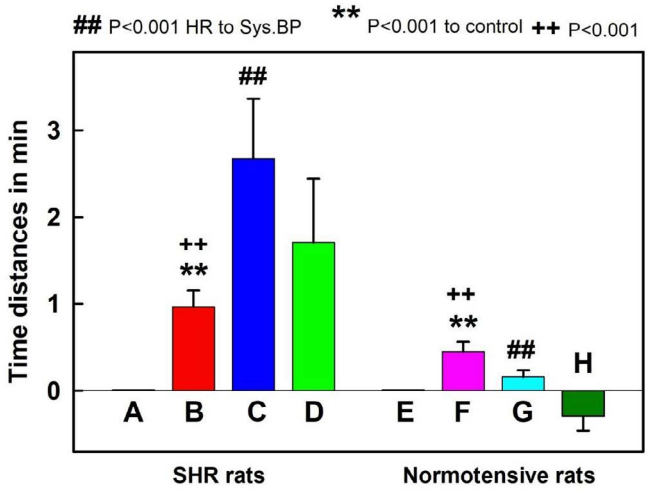


Figure 5: Comparison of delayed effects of GSNO (80 and 32 nmol/kg, respectively) in SHR and NORM rats in min. Data were taken from Figures S12 and S13. Time of GSNO administration was set to zero (A,E). Time of the transient Sys.BP maximal decrease in SHR (B) and NORM (F) rats. Time of the transient HR maximal decrease in SHR (C) and NORM (G) rats. Time between transient maximal HR decrease and transient Sys.BP decreased in SHR (D) and NORM (H) rats. Means ± SD, n=7. Significance t-test, P < 0.001; ** B vs A or F vs E; ## C vs A or G vs E; ++ C vs B or G vs F

To study more details of the GSNO effects, its influence on possible 'n-gital' APW-Ps properties was evaluated. The temporal position of Sys.BP on APW has been found not to gradually shift on the waveform in response to increasing/decreasing vascular stiffness, as it was expected, but it showed di-gital, tri-gital, and tetra-gital (n-gital) time fluctuations [11]. Since GSNO decreases vascular stiffness, I used similar APW-Ps to determine whether GSNO induces n-gital fluctuations of some APW-Ps in SHR rats. Time-dependent effect of GSNO on n-gital APW-Ps is shown in Figures 6, S3D, S4D-S9D. Based on studies of [11] I concentrated on two APW-Ps, (u) which is detecting di-gital time position of Dia.BP on APW and parameter (w) which is detecting di-gital, tri-gital, and tetra-gital time positions of Sys.BP on APW. After administration of GSNO during decreasing Sys.BP parameter (w) n-digitally decreased from L-4 level through L-3 and L-2 levels to basic L-1 level. After Sys.BP later gradually increased, parameter (w) increased n-digitally from L-1 through L-2 and L-3 to L-4 level. Similar results were observed from parameter (v). The parameter (u) that detects the digital time position Dis.BP was mostly in an activated D-2 position and randomly transitioned to the basic D-1 position, with the time interval between the D-2 and D-1 positions not being constant. Similar results were observed in four experiments Figures S4D-S7D. In two experiments, n-gital time fluctuation was not observed due to the less pronounced effect of GSNO, which reduced Sys. BP to only ~140 mmHg (Figures S8D-S9D). In Exp-1, it was observed that level L-4 was divided into two sublevels, L-4-a and L-4-b (Figure S3D). In some experiments APW-Ps, which measure distance between anacrotic notch (AnN) and Dias.BP (s) and/or distance between AnN and dP/dtmax (t) showed di-gital fluctuation (Figures S4D-S9SD). Since the fluctuation frequency was similar to that of a rat's breathing and the fluctuation amplitude was ≤ 3 ms, it is unclear yet whether these parameters reflect the digital movement of the AnN on the APW [11].

Discussion

Our study of the detailed time-dependent effects of GSNO on APW in SHR rats and comparison to NORM rats provides a wealth of original information characterizing cardiovascular system. The results presented here show that the effect of GSNO on APW-Ps is not universally applicable to rats under various conditions, and suggest that this is likely true for other animals and humans as well. Our discussion is focused mostly on interrelationships between APW-Ps. NO signaling pathways in the cardiovascular system have been thoroughly investigated at molecular level [3, 4], but connections/disconnections among NO pathways are not yet fully understood. Based on the interrelationships between APW-Ps in SHR rats, I indentified at least 13 of 43 hemodynamic APW-Ps, which are differently regulated by NO than is regulated Sys.BP. It should be noted that, just as the interactions between 43 APW-Ps and Sys.BP are presented, the interactions between the remaining 43 APW-Ps could also be evaluated. In total, there are 946 interrelationships between two of the 44 APW-Ps. In this study, only 4.5% of these interrelationships were evaluated. Since I have identified a number of new interrelationships, I needed to further narrow the scope of our discussion. Of the 13 interrelationships in total, I focus only on the effect of GSNO on HR and Sys.BP and compared them in SHR and NORM rats (Figures 5, S12, S13). It is noticed that Figures 2, 4, S11 show a 2-dimensional interrelationships only, time dimension is not shown. The time dimension could be obtained if three-dimensional graphs are constructed. However, I found them too complex for visual presentation. Concentration of NO in basal physiological or activated conditions is within 0.1 – 1000 nmol/L [18]. A rat weighing 300 g has a blood volume of about 20 ml and a cardiac output of approximately 20 ml per 15 seconds. From this, I can estimate that when 160 µmol/L of GSNO was i.v. administered for 15 s, its initial concentration in the blood was 1.2 µmol/L and 80 nmol per kg body. Taking into account half-life of NO in organism around 0.1 to 2 s and in blood even < 1 s [19, 20], the rapid effect of GSNO on Sys.BP and HR (< 30 s) can be attributed to the released NO from GSNO, which interacts immediately with receptors on the membranes of the heart and blood vessels (Figures S12, S13). I cannot explain the differing or even opposite rapid effects observed in SHR and NORM rats in some APW-Ps (Figure S10) and particularly in HR (Figures S12, S13), but I hypothesize that these rapid NO signaling pathways differ

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in these animal models at different physiological conditions, and/or different signaling pathway are activated at different NO concentrations.

Our data revealed the transient pronouncedly delayed effect of GSNO in SHR rats to decrease Sys.BP (~ 1 min) and HR (2.7 min) suggesting that there are different 'delayed signaling pathways' regulating Sys.BP and HR. This is valid also for delayed effect of GSNO in NORM rats even it was significantly shorter in comparison to SHR rats (Figure 5). From the comparison of the delayed values for SHR and NORM rats it is suggested that the 'delayed signaling pathways' are different in these two animal models and different from the rapid signaling pathways. I do not know a basic of the delayed responses resulted probably from interactions of NO with blood and cell component, which can also include central and peripheral nervous systems [21], baroreceptors, visceral afferents [22], cardiovascular reflexes [23], cardiac autonomic ganglia [24] or effects on transmembrane currents [25].

In our study, the time dependent HR fluctuated irregularly up/down immediately after GSNO administration, but later the overall effects was transient decrease of HR, which is opposite that have been reported using different experimental model and different NO donors. Hogan et al. [26] reported HR increase after i.v. administration of NO donor molsidomine in vagotomized rabbits. NO donor sodium nitroprusside increased heart rate evaluated in sinoatrial node/atria preparations [27]. NO donors sodium nitroprusside or 3-morpholinosydnonimine increased beating rate in isolated guinea pig sinoatrial node/atrial preparations at nmol/L to µmol/L, but decreased it at mmol/L concentrations [25]. I assume that the differences between our results and the published data may stem from the time frame during which HR changes were assessed. The results stress high complexity of interactions of NO in HR regulation [3]. Unexpected opposite dependences of the same APW-Ps observed from a comparison of interrelationships between APW-Ps in NORM and SHR rats (Figures 4, S11–S13) show that there is still much to be learned about NO pathways. The physiological basis for these 'opposite' results is not yet clear.

Each of the interrelationships (Figures 2, 4, S11–S13) reflects a connection between signaling pathways regulating the two particular APW-Ps. Each plot of the two APW-Ps is the 'signaling pattern' for the specific cardiovascular condition, and some of them may be unique for changes of NO concentration in the cardiovascular system. The interrelationships among the APW-Ps provide additional important characteristics of the cardiovascular system that

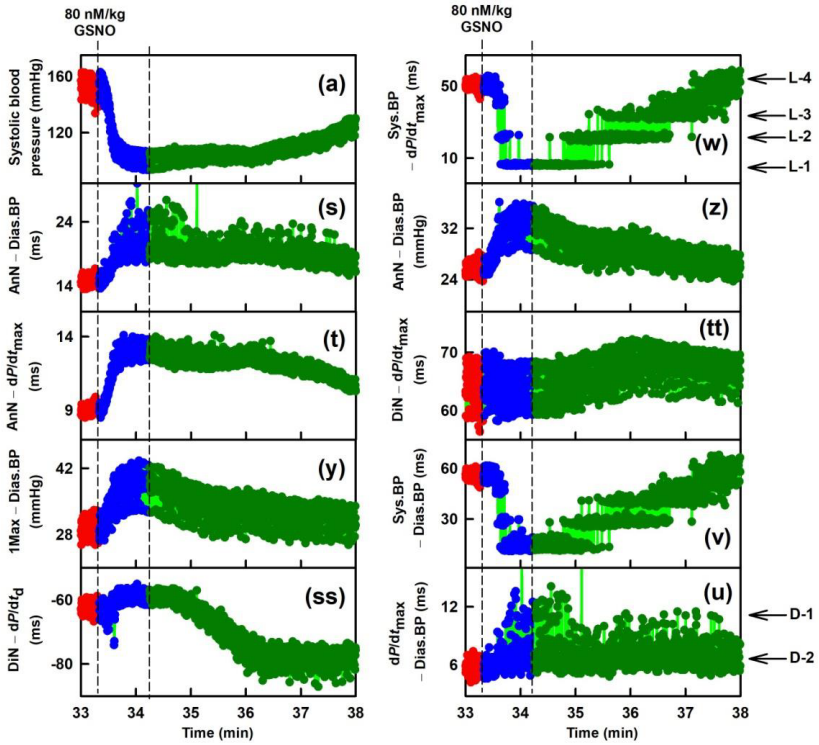


Figure 6: Exp-1. Time-dependent changes in ten APW-Ps at control and after bolus administration of 80 nmol/kg GSNO of anesthetized rat. Red, blue, and dark green dots are individual heartbeats, which are mostly not resolved at this low resolution. The bright green lines show the connection between adjacent heartbeats. Control (red), decreasing (blue) and increasing (dark green) Sys.BP after GSNO administration. Vertical dashed lines indicate administration of GSNO (left dashed line) and minimal value of Sys.BP (right dashed line). Horizontal arrows indicate predicted D-1 and D-2 (di-gital) levels and L-1 to L-4 (n-gital) levels [11]. Definitions, units, and abbreviations of APW-Ps evaluated from APW are explained in Figure S1

can be attributed to its specific conditions. In the future, these parameters may serve as a database characterizing the cardiovascular system in both physiological and pathological states [5,12-14]. Artificial intelligence will be needed to analyze and assist in drawing conclusions from the vast amount of cardiovascular data obtained from APW-Ps and their interrelationships [28-29]. Our study of GSNO effects on n-gital fluctuation of some APW-Ps confirmed that GSNO, which decreases vascular stiffness, induced not constant di-gital fluctuation of Dias.BP and constant di-gital, tri-gital, and tetra-gital time fluctuations of the temporal position of Sys. BP on APW (Figure 6). The results, under these experimental conditions, are similar that found for other compounds which transiently decreased vascular stiffness [10].

Conclusion

The observed effect of GSNO on 44 APW-Ps of SHR rats and on n-gital fluctuation of temporal position of systolic BP has reinforced the hypothesis that it is possible to characterize cardiovascular system in many patho-physiological conditions just from the detail shape of APW. Based on the interrelationships between APW-Ps in SHR rats, I indentified at least 13 of 43 hemodynamic APW-Ps, which are differently regulated by NO than is regulated systolic BP. Comparison of heart rate interrelationships with systolic BP under transient GSNO concentrations in SHR and normotensive rats revealed that their 'signaling pathways' are significantly different and even produce opposite results. The results revealed that the effect of GSNO on APW-Ps is not universally applicable to rats under various conditions, and suggest that this is likely true for other animals and humans as well. I confirm that APW is highly sensitive to subtle changes in the cardiovascular system and that APW-P and their interrelationships are suitable for investigating their association with specific states of the cardiovascular system. The APW-Ps can provide patterns for particular cardiovascular conditions and their interrelationships can provide information of 'signaling patterns' leading to better understand signaling physiology. From a clinical perspective, this approach may be used in animal models and in humans to create a data bank of APW-Ps and their interrelationships patterns for different cardiovascular conditions.

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Author Contribution: Karol Ondrias: Writing – original draft, Supervision, Methodology, Investigation, Data curation, Validation, Conceptualization, Funding acquisition.

Ethics approval statement

The Central Animal Housing Facility of Pavilion of Medical Sciences (registration number SK UCH 04022) and animal housing facility of the Institute of Normal and Pathological Physiology (registration number SK UCH 03018) both operating by Centre of Experimental Medicine (Bratislava, Slovak Republic) provided the veterinary nursing care. All procedures used were approved by the Ethic Committee of the Institute of Normal and Pathological Physiology and the Ethic Committee of the Biomedical Research Center and were acknowledged by the State Veterinary and Food Administration of the Slovak Republic (C.k. Ro-6023/2022–220 and Ro 5428–3/2021–220) using the guidelines from Directive 2010/63/EU of the European Parliament. All animal experiments complied with the ARRIVE guidelines.

Informed consent: Not applicable

Appendix A. Supplementary information

Supplementary information to this article can be found online

Data availability

Data are available from the corresponding author upon reasonable request.

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