Experimental Physiology

Organ-specific ligation-induced changes in harmonic components of the pulse spectrum and regional vasoconstrictor selectivity in Wistar rats

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It has been shown previously that the amplitudes of the harmonic components of the pulse spectrum vary in specific patterns when the arteries leading to different organs are ligated, with the variations in the harmonics being linearly additive. Since ligation can be regarded as a vast increase in organ resistance, the present study examined the potential of using these ligation-induced variations in the pulse spectrum as reference parameters for an increase in vascular resistance and for regional vasoconstrictor selectivity. A vasoconstrictor, either arginine vasopressin (AVP) or angiotensin II (Ang II), was infused into anaesthetized Wistar rats via the femoral vein for 1 h. The distinct harmonic-specific drug effects on the pulse spectrum were simulated by combining renal artery and superior mesenteric artery ligations in different ratios, the ratio with the lowest mean square difference determining the regional drug selectivity. The ratios indicated that the effect of AVP on the pulse spectrum was attributable to the combined effect of ligating the renal and superior mesenteric arteries, while the effect of Ang II was attributable to ligation of the renal artery. The results are comparable with those of investigations of regional vascular resistance performed using traditional methods. Our findings indicate that the ligation-induced variations in the pulse spectrum can be used to determine regional increases in vascular resistance. This implies that blood pressure can be used as the sole parameter to determine which arterial bed has been affected by the vasoconstrictor, and how seriously.

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The blood pressure wave contour has long been studied as a putative non-invasive tool with which to reveal problems in the circulatory system. The influence of the reflective wave on the pressure wave contour due to the change in wave velocity under different physical situations has been examined extensively (O'Rourke *et al.* 2001). The augmentation index (Murgo *et al.* 1980; Kelly *et al.* 1989; Nicholes & Singh, 2002) or the second derivative of the waveform (Takazawa *et al.* 1998) provides more information about changes in vascular load than either the systolic pressure (SP) or the diastolic pressure (DP). However, using the pressure wave to analyse the behaviour of the vascular bed is still far beyond the scope of these indices; other vascular-bed-related pulse analysis parameters need to be determined.

Resonance theory

The pattern of harmonic components of the arterial-bed-specific pulse spectrum might be suitable for analysing the behaviour of the vascular bed. According to resonance theory (Wang *et al.* 1991; Yu *et al.* 1994; Jan *et al.* 2003), each arterial bed in the vascular system is forced to oscillate by pressure waves at its own resonant frequency. As the physical properties of an arterial bed change, its resonant frequencies will also vary. However, the same resonance harmonics will be maintained by the heart rate (HR) control system to minimize energy loss (Jan *et al.* 2003). The amplitudes of the resonance harmonics change more than other harmonics. Since the pulse spectrum is the combined result of the influence from all arterial beds, we

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may therefore deconstruct it into components that relate to specific arterial beds.

Evidence for arterial-bed-specific harmonic components of the pulse spectrum

Farrow & Stacy (1961) found that clamping the femoral artery produced large variations in only the third harmonic of the pressure wave, and Wiener *et al.* (1966) reported that the pulmonary circulation had the strongest effect on the fourth harmonic. Wang *et al.* (1989) and Young *et al.* (1989, 1992) reported that ligating the renal, gastric, splenic or superior mesenteric artery for a very short duration (less than 3 s) caused specific changes to individual harmonics. Young *et al.* (1989) further proved that the organ ligation effects were linearly additive; ligating two different arterial beds simultaneously (the renal artery and the superior mesenteric artery) changed the pulse spectrum in a similar manner to the direct addition of the two spectra resulting from individual ligations. This suggests that these linearly additive organ ligation spectra can be used as independent parameters to elucidate the physical status of specific vascular beds.

Organ ligation spectra and regional drug selectivity

The regional drug selectivity of different vascular beds is an important issue in pharmaceutics. Traditional methods of evaluation require both blood pressure and flow data. Often this involves highly invasive surgery in order to place several probes on different arteries of the test subject, which certainly disturbs the circulatory system. However, the artefacts may be greatly reduced if the regional selectivity could be revealed by performing pressure measurements only. Since the effect of ligation can be regarded as a huge increase in the resistance of the ligated vascular bed, increase in vascular resistance during vessel constriction can be considered to be equal to ligating the arterial bed by a certain amount. The potential of using variations in organ ligation spectra as reference parameters to study the regional selectivity of vasoconstrictors was examined in this study.

Simulation method

The chemical ligation effects of two potent vasoconstrictors, arginine vasopressin (AVP) and angiotensin II (Ang II), were studied. These two vasoconstrictors reportedly exhibit different selectivities between the renal and mesenteric vascular beds (Hellebrekers *et al.* 1990; Jackson & Herzer, 2001). We simulated the drugs that affected pulse spectra by combining the effects of renal artery ligation and superior mesenteric artery ligation in different ratios. The best ratio (with the least mean square difference) was obtained by computer analysis, and the associated regional drug selectivity was compared with results obtained by traditional methods (Hellebrekers *et al.* 1990; Jackson & Herzer, 2001).

Methods

Animal preparation

The investigation conforms with the "Guide for the Care and Use of Laboratory Animals" published by the US National Institute of Health. Male Wistar rats weighing between 240 and 320 g were obtained from the Experimental Animal Center of National Taiwan University, Taipei, Taiwan. The animals were housed in our animal care facility with a 12 h:12 h light–dark cycle for at least 1 week before experiments were conducted. They received water and food *ad libitum* (Labdiet 5001 Rodent diet, PMI Nutrition International, Brentwood, MO, USA) prior to the experiments.

The rats were divided into three experimental groups: AVP infusion (5 pmol min^{-1}, $n = 14$), Ang II infusion (20 pmol min^{-1}, $n = 10$) and saline infusion (control group, 5 μl min^{-1}, $n = 9$). Both drugs were dissolved in saline, with the doses chosen so that the two drugs would induce the same increase in DP after 1 h of continuous infusion at a rate of 5 μl min^{-1}. AVP and Ang II were purchased from Sigma (St Louis, MO, USA).

The rats were anaesthetized with urethane only (1.1 g kg^{-1} 2 ml^{-1} in saline, I.P.) (Sigma, St Louis, MO, USA) with a heat pad to keep the body temperature. The femoral vein was exposed through an inguinal incision and a polyethylene cannula (PE-10, PE-10 I.D. 0.28 mm, O.D. 0.61 mm) was inserted. Drugs were infused through the polyethylene tubing at the doses mentioned above, at a rate of 5 μl min^{-1} using a pump (Gilson minipuls 2, Gilson Medical Electronics, Middleton, WI, USA). The tail artery was cannulated with an intravenous catheter (Angiocath Plus, 22 GA needle size, 1.00 IN needle length, 0.9 × 25 mm, Becton-Dickinson, Seoul Korea) filled with physiological saline and heparin 1.5 kg g^{-1} 2 ml^{-1} in saline, which was then connected to a pressure transducer (P10EZ, Ohmeda, Singapore). The cannulation opening on the tail artery was about 1 cm from the anus. The aortic blood pressure pulse then passed through the catheter tip (25 mm from the opening) to the pressure transducer.

After all recording procedures had been completed, the rats were killed by overdose urethane.

Recording procedure

The pressure signal was sent to a preamplifier (Universal amplifier, Gould Instrument Systems, Valley View, OH, USA) and then to a 16 bit A–D converter PC interface card (AX5621, Axiom Technology, Hsin Tien, Taiwan) for data analysis.

After all of the operations were completed, at least 3 h was allowed for stabilization. A 1 s data sequence (5–8 pulses) was sampled every minute, with 20 data sequences (about 140 pulses) being recorded over 20 min as self-controlled data. Three criteria were employed to ensure that the control data sampling was sufficiently accurate, representative and stable: the coefficients of variance (CV, s.d./mean) of the HR, the DP and the amplitudes of the first two harmonics of the pressure wave were all required to be less than 2%. Where the CV values exceeded this limit, the animal was considered unstable and data were discarded.

After the control period, drug or saline was infused continuously into the femoral vein at a rate of 5 μl min^{-1} (AVP at 5 pmol min^{-1}, Ang II at 20 pmol min^{-1}) for 1 h. The effects during and 1 h after drug infusion (two sets of 60 1 s pulse sequences) were recorded.

Data analysis

Since we were mainly interested in the relative amplitudes of each harmonic in the blood pressure spectrum, only the pulsatile parts of the pressure waves were calculated. We separated each pulse in a pressure wave sequence at its two lowest points (the place where two neighbouring SP waves started). Every isolated pulse was then Fourier transformed into the frequency domain. For each pressure pulse, the amplitude A_n (for harmonics $n = 1$–6) was normalized by the mean level of the pulse A_0, and the harmonic proportions (HPs) for each harmonic C_n were calculated as percentages using $C_n = 100(A_n/A_0)$.

The entire experiment was divided into seven time periods, each with 20 1 s pulse sequences: the self-controlled period (from −20 to 0 min), three drug-infusion periods (0–20, 20–40 and 40–60 min) and three after-drug periods (60–80, 80–100 and 100–120 min). We defined the drug effect as the percentage difference in the proportion of the nth harmonic:

$$(\%\text{Diff\_HP}) = \Delta C_n = 100(C_{dn} - C_{cn})/C_{cn},$$

where C_{cn} is the averaged HP of the nth harmonic of all of the pulses in the self-controlled period, and C_{dn} is the averaged HP of the nth harmonic of all the pulses in the during- and after-drug-infusion periods.

Since the amplitudes decreased rapidly with the harmonic number, we considered only the first six harmonics (i.e. $n = 1$–6).

Statistical analysis

Student's paired t test was used for statistical comparisons. The drug effects on the HP, DP, SP and HR during and after drug infusion were compared with the control values. The drug effects were considered to be significantly different from the control values if $P < 0.001$. For HP, the averaged %Diff_HP values were considered to be significantly different from zero if $P < 0.001$.

Analyses of variance were not performed because differences between groups were not of interest in this study.

Chemical ligation simulation

The superior mesenteric artery ligation effects $(\%\text{Diff\_HP})_m$ and the single-side renal artery ligation effects $(\%\text{Diff\_HP})_r$ reported by Young *et al.* (1989) were combined at different ratios (R_r and R_m) to simulate the effects of AVP and Ang II, respectively, according to the following equation:

$$(\%\text{Diff\_HP})_{combined} = R_r(\%\text{Diff\_HP})_r + R_m(\%\text{Diff\_HP})_m.$$

The summation of the square of the difference (SSD) between $(\%\text{Diff\_HP})_{drug}$ and $(\%\text{Diff\_HP})_{combined}$ was calculated for the first to the sixth harmonic as:

$$\text{SSD} = \Sigma\text{square}[(\%\text{Diff\_HP})_{drug} - (\%\text{Diff\_HP})_{combined}]_n.$$

where $n = 1$–6.

The best simulation effect was obtained by computer searching for the minimum value of SSD with both R_r and R_m ranging from 0 to 10 in 0.01 increments.

Results

The averaged DP, SP and HR in each time period for the three groups are shown in Fig. 1. Saline had no significant effect on DP, SP or HR during any of the time periods, but both drugs caused significant increases in DP and SP, and a significant decrease in HR. There was a rebound in the HR as the effects of AVP faded, but no such rebound effect was found for Ang II.

The effects of saline, AVP and Ang II on the blood pressure pulse spectrum are presented in Fig. 2. The average percentage differences in harmonic proportion (%Diff_HP) of the six during- and after-infusion periods are shown in the figure.

Figure 3 shows the characteristic variations in the spectra induced by each drug. It can be seen that %Diff_HP did not differ significantly from zero during the first 40 min infusion period in the saline group for all six harmonics and that the variations in the spectrum were not specific, indicating no difference between the during- and after-saline-infusion periods. However, there was a small decrease in C_2 and C_3 during the continual infusion period, and the effects were sustained to the after-saline-infusion period.

AVP caused significant decreases in C_1, C_2, C_4, C_5 and C_6 during the three drug-infusion periods. The effects on C_1, C_4, C_5 and C_6 were sustained for 20 min after drug infusion. The effects of AVP on C_2 recovered quickly, and those on C_5 and C_6 lasted longer than those on the other

harmonics. There is a clear peak in C_3 in the three AVP-infusion curves, but not in the after-AVP-infusion curves.

Angiotensin II caused significant decreases in C_3, C_4 and C_5 during the three drug-infusion periods. These effects diminished soon after the drug infusion was stopped. It is also evident that the effect on C_2 increased with the infusion time. The effects became significantly different from zero from the 40–60 min time period until the end of the experiments. The characteristics of the variations in the spectrum faded after the infusion of vasoconstrictors.

In Fig. 4, the experimental effect of drugs and the simulated chemical ligation effects are plotted against the blood pressure spectrum; the two curves are similar. %Diff_HP decreases with the harmonic number for Ang II curves, whereas a relative peak at the third harmonic and a minimum at the fifth harmonic are evident for AVP.

From the best-fit data, the ligation ratio ($R_r : R_m$) is 0.41 : 0 for Ang II and 1.05 : 0.47 for AVP. Angiotensin II had little effect on the superior mesenteric vascular resistance.

Discussion

Main achievements

This study demonstrates that variations in the harmonics of the pulse spectrum can be used to identify the vasoconstrictor affected internal organs. Using only the pressure spectrum, we found that the effects of Ang II can be attributed to renal artery ligation (Young *et al.* 1989, 1992; Wang Lin *et al.* 1991; Jan *et al.* 2003), whereas AVP is more likely to affect both the renal and superior mesenteric arteries (Young *et al.* 1992). The best-fit ligation ratio for Ang II (Fig. 4) indicates that it induced a resistance change of about 20% from each kidney but had little effect on the superior mesenteric vascular resistance. In contrast, AVP induced a resistance change of about 50% from each kidney as well as 4.7 % in the superior mesenteric bed.

In this preliminary study, we minimized the anaesthetic dosage and avoided possible surgical interference to the blood pressure spectrum. We did not measure the local vascular resistance, but instead compared our data with the distinct regional vascular selectivity studies on AVP and Ang II by Jackson & Herzer (2001). They reported that intravenous infusion of AVP caused similar increases in the vascular resistance in renal, mesenteric, carotid, hindquarter and other tissues. Angiotensin II, however, caused a noticeable increase in renal vascular resistance, with lesser effects on mesenteric vascular resistance, little effect on carotid vascular resistance, and no effect on hindquarter or other tissue vascular resistance. Similar effects in dogs have been reported by Hellebrekers *et al.* (1990). These reports coincide well with our data and support our claim that the effects of vasoconstrictors on regional vascular selectivity can be determined using only measurements of the blood pressure spectrum.

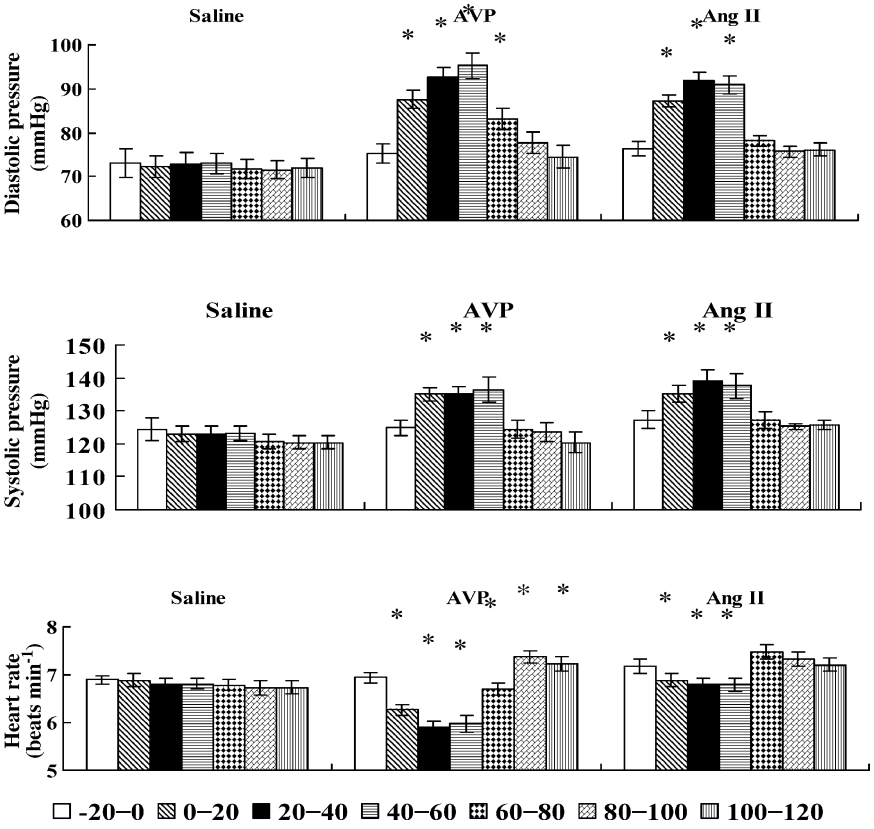

Figure 1. Effects of saline ($n = 9$), AVP ($n = 14$) and Ang II ($n = 10$) on DP, SP and HR
The error bars indicate S.E.M. * Significant difference ($P < 0.001$) between the controls and the drug-infusion effects during each during- and after-infusion time periods.

Influences from blood pressure and heart rate changes

Young *et al.* (1989, 1992) found that the changes in HR and mean blood pressure during organ ligation were insignificant. A very short duration of ligation (less than 3 s) avoided possible physiological reflexive responses; variations in the pulse spectrum due to organ ligation were only related to the intrinsic physical properties of each organ.

In the present study, however, both vasoconstrictors significantly changed the mean blood pressure and the HR. The autoregulatory effect due to the increase in blood pressure (Hellebrekers *et al.* 1990; Jackson & Herzer, 2001) and the influence of shifts in harmonics due to HR changes can be summarized as follows.

First, both drugs caused very similar changes in the blood pressure and HR, suggesting that the influence of blood pressure and HR on variations in the pulse spectrum should be about the same. Hence, the distinct drug-affected variations should be related mainly to the regional drug selectivity.

Second, the kidney reportedly has the highest autoregulatory capability, and so the autoregulatory effect initiated by the increase in blood pressure might account for the smaller decrease in C_2 elicited by both drugs.

Third, the effects of AVP and Ang II on the harmonic components of the pulse spectrum should be less than the 10–20% changes in HR evident in Fig. 1. For a periodical signal such as a pressure pulse, most of its energy is concentrated into its harmonic bands (Jan *et al.* 2003). Therefore, the frequency-dependent behaviour of the vascular system should be related to the harmonics of the HR rather than the intrinsic resonant frequencies to ensure optimal energy usage. When AVP or Ang II changes the physical properties of the affected vascular beds, thus changing their resonant frequencies, the heart should adjust its pumping rate to keep the pressure energy focused at the new harmonic bands, and so the specific relationships between the organs and the harmonics should be maintained. Therefore, despite the changes in the frequency of each harmonic, we may still compare the two pulse spectra on the basis of the harmonic components due to the maintained relationship between the organs and the harmonics.

Influences of flow changes

Changes in the input flow to the vascular bed during drug infusion would have had little effect on our analyses for the following reasons.

First, the amplitude of each harmonic in the pressure pulse was normalized by the mean level of the pulse, which avoids any possible cardiac influence.

Second, the pulse spectra were compared on the basis of harmonics rather than their actual frequencies. Therefore, the comparisons are related to the resonance status of the vascular system and not to the vascular

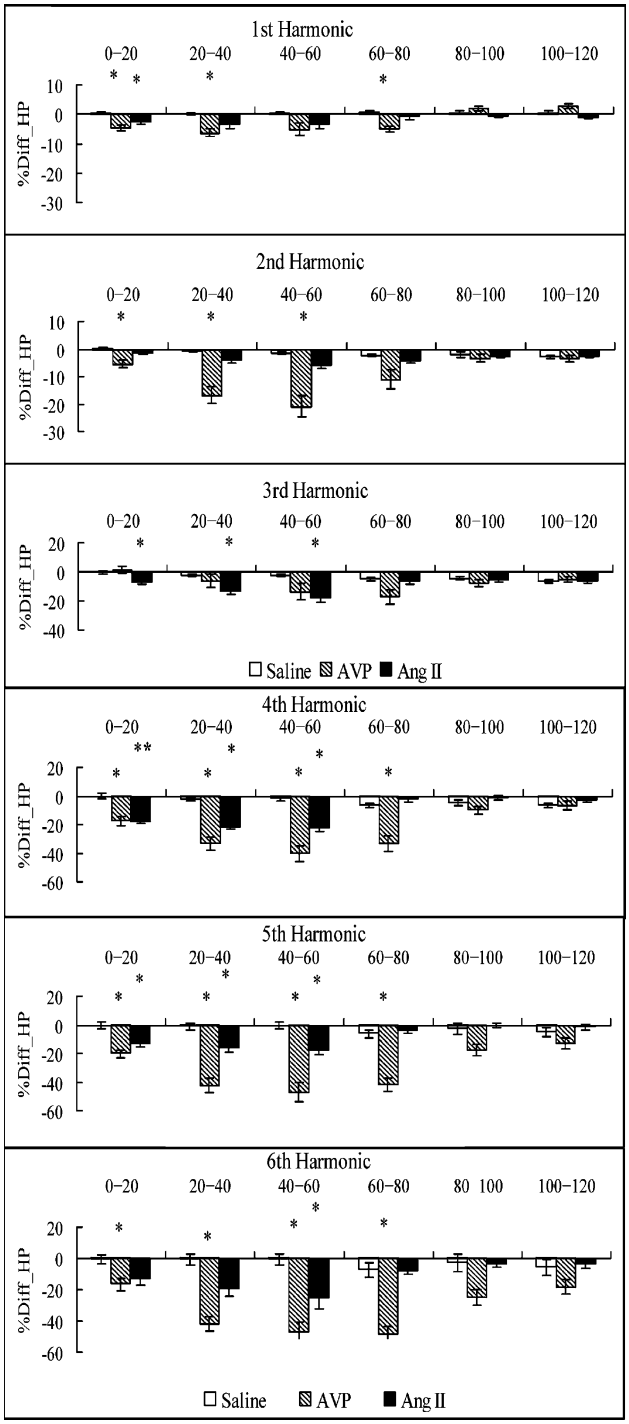

Figure 2. Effects of saline ($n = 9$), AVP ($n = 14$) and Ang II ($n = 10$) on the blood pressure spectrum for the first 6 harmonics

The average percentage differences in %Diff_HP during each during- and after-infusion time period are shown. The error bars indicate standard errors. * Indicates that %Diff_HP is significantly different ($P < 0.001$) from zero, indicating that the drug effect is significantly different from the control.

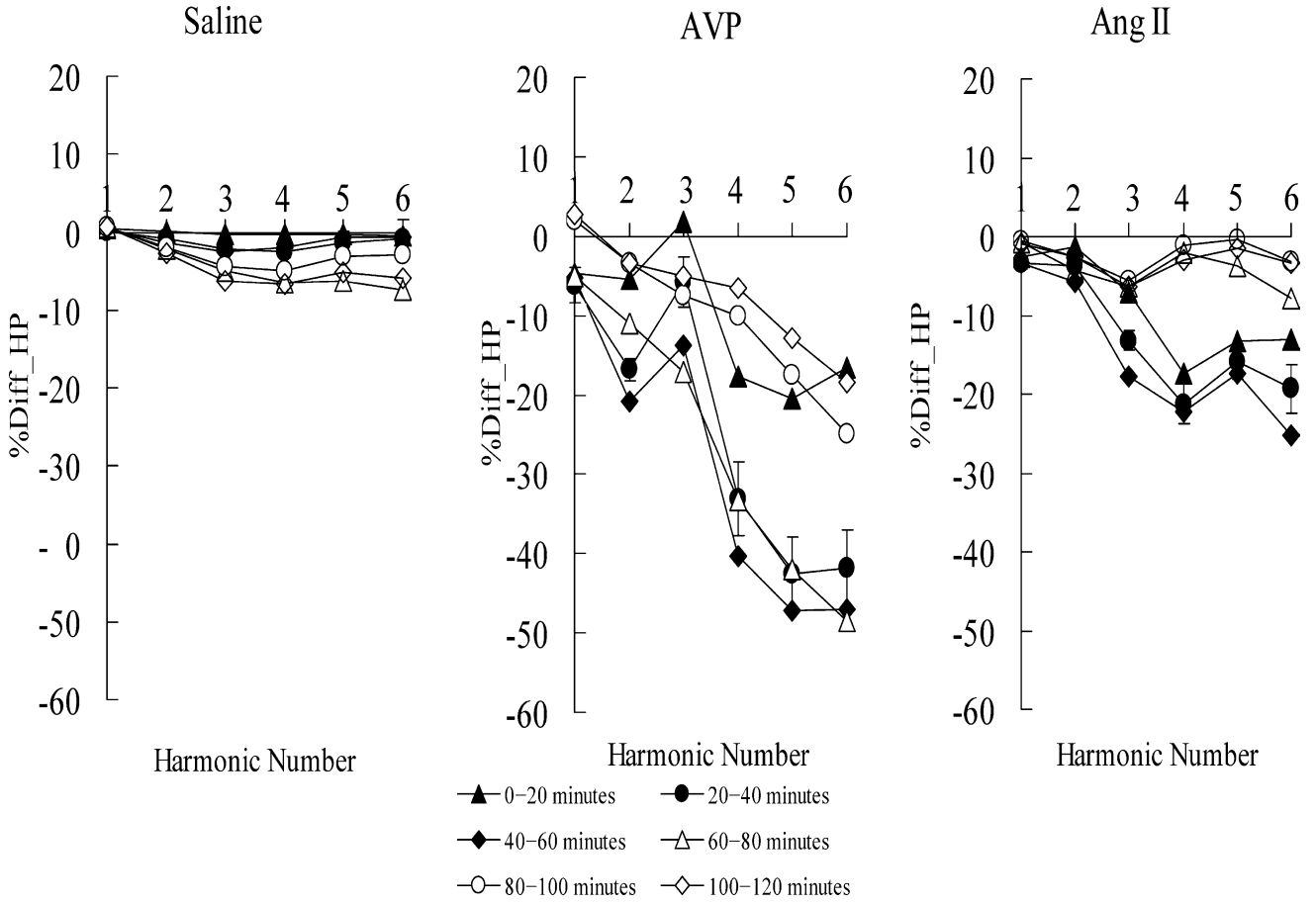

Figure 3. Characteristic variations in the pulse spectrum caused by infusions of saline (left), AVP (middle) and Ang II (right)
The average percentage difference in %Diff_HP is plotted *versus* the harmonic number for the first 6 harmonics. There are 3 during-drug-infusion periods (0–20, 20–40 and 40–60 min) and 3 after-drug periods (60–80, 80–100 and 100–120 min).

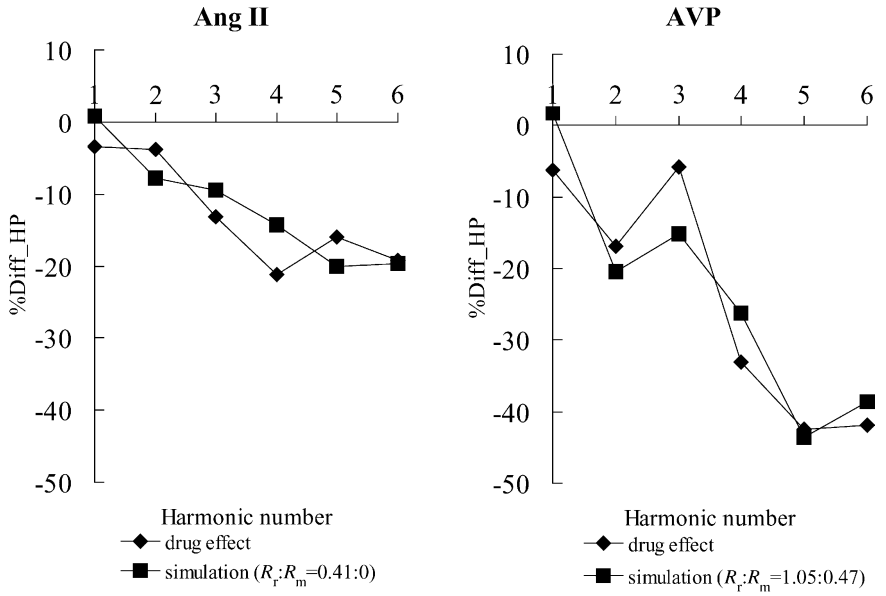

Figure 4. Measured drug effects on the blood pressure spectrum
The left panel shows the effects of 20 pmol min^{-1} Ang II at 20–40 min. The right panel shows the effects of 5 pmol min^{-1} AVP at 20–40 min. The figure also shows their simulated chemical ligation effects.

impedance or the input flow. In this study, the ligation profile or the drug effect profile describes how the vascular resistance changes, and not how the impedance changes.

Influences from the occluded tail artery

To avoid possible surgical interference on the blood pressure spectrum, we chose fluid-filled catheters rather than micromanometers for the pressure measurements. Compared with other surgical techniques, opening the tail artery was considered to have the least influence on the vascular system, and the self-control design of the study minimized the possible influence from tail artery occlusion as well as the damping effect.

Further improvements

First, our method provides relative vasoconstrictor selectivity information from the ligation data of two vascular beds. The calculations only account for the two largest arterial beds, since there are few experimental data on the smaller arterial beds. Further experimental data should therefore be collected.

Second, the vascular reactivity may differ with the animal species or anaesthetic used (Faber, 1989). Different experimental set-ups may also affect the results (Lappe & Brody, 1984; Gardiner *et al.* 1988, 1989). Quantitative comparisons performed under similar conditions between the regional vascular resistances and the ligation ratios may further improve the similarity.

Possible applications

This study forms part of a series aimed at proving that the blood pressure waveform can be used as the sole parameter to determine the blood circulatory condition of the arterial bed (Wang *et al.* 1989, 1996; Young *et al.* 1989, 1992; Wang *et al.* 1991, 2000; Lu *et al.* 1996; Jan *et al.* 2003; Hsu *et al.* 2003; Lin Wang *et al.* 2004). It is easy to obtain pulse information in humans non-invasively from the radial artery or several other points on the body. The pulse analysis method has the potential to be developed as a non-invasive quantitative monitoring tool (Lu *et al.* 1996; Hsu *et al.* 2003; Kizilova, 2003). To achieve this, the frequency characteristics of more human vascular beds must be determined. The potential clinical relevance is presently limited by the need for training data sets. The applicability of the method is as follows.

First, the resonance theory is based on large arterial beds, such as the major internal organs, having unique shapes, sizes and structures, which results in each bed having its own resonant frequency that causes its own specific ligation profile (Wang *et al.* 1989; Wang *et al.* 1991, 2000; Jan *et al.* 2003; Lin Wang *et al.* 2004). We have presented the specific frequency characteristics of six large arterial beds in the Introduction, each of which has a distinct profile (Farrow & Stacy, 1961; Wiener *et al.* 1966; Wang *et al.* 1989; Young *et al.* 1989, 1992). There is also indirect evidence supporting these suggestions from drug studies (Wang *et al.* 1995, 1997, 2003; Hsu *et al.* 2003) and clinical observations (Chen *et al.* 1993; Wang *et al.* 1996; Lu *et al.* 1996).

Second, the application of the law of linear superposition to the pressure spectral pattern is another key aspect of our method. Milnor (1989) stated that many findings support the conclusion that the arterial system is approximately linear (up to 98%) with respect to impedance. So the linearity of the pressure spectral pattern for most major arterial beds is expected. We have also observed that the pressure spectra were approximately linear in our previous herbal studies (Wang *et al.* 1995, 1997, 2003).

Third, the ligation data can only mimic the increases in local vascular resistance, because both events (ligation and vasoconstriction) cause the vascular system to deviate from its original state in a similar way. Conceptually, it is not adequate to consider ligation as negative vasodilatation on a resonance basis, and hence this ligation-data method is limited to vasoconstrictors. We therefore need other pulse analysis parameters that mimic the decreases in vascular resistance to evaluate the effects of vasodilators.

Conclusions

Many studies have shown that the blood pressure wave contour may reveal more valuable internal physiological information than that of SP and DP alone. The present study strengthens this concept by demonstrating that pressure-wave spectrum analysis reveals not only which arterial bed is vasoconstictor affected but also the magnitude of the change. We were able to detect the changing ratios of the vascular resistance, and so the regional AVP and Ang II selectivity, from distinctive changes in the blood pressure spectrum.

Conventional investigations of vascular resistance provide detailed data on regional blood flow and pressure by placing multiple monitoring probes inside the body via surgical approaches. The proposed non-invasive pulse analysis method provides a complementary macroscopic summary of regional selectivity.

References

Chen CY, Wang WK, Kao T, Yu BC & Chiang C (1993). Spectral analysis of radial pulse in patients with acute, uncomplicated myocardial infarction. *Jpn Heart J* **34**, 131–142.

Faber JE (1989). Effects of althesin and urethan-chloralose on neurohumoral cardiovascular regulation. *Am J Physiol* **256**, R757–R765.

Farrow RL & Stacy RW (1961). Harmonic analysis of aortic pressure pulses in the dog. *Circ Res* **9**, 395–401.

Gardiner SM, Bennett T & Compton AM (1988). Regional haemodynamic effects of neuropeptide Y, vasopressin and angiotensin II in conscious, unrestrained, Long Evans and Brattleboro rats. *J Auton Nerv Syst* **24**, 15–27.

Gardiner SM, Compton AM & Bennett T (1989). Regional haemodynamic effects of vasopressin infusion in conscious, unrestrained, Brattleboro rats. *Br J Pharmacol* **97**, 147–152.

Hellebrekers LJ, Liard JF, Laborde AL, Greene AS & Cowley AW Jr (1990). Regional autoregulatory responses during infusion of vasoconstrictor agents in conscious dogs. *Am J Physiol* **259**, H1270–H1277.

Hsu TL, Chiang Y, Wang WK, Chao PT, Bao JG & Wang Lin YY (2003). Pulse analysis as a possible real-time biomarker complementary to SGPT and SGOT for monitoring acute hepatotoxicity. *Toxicol Mechanisms Meth* **13**, 181–186.

Jackson EK & Herzer WA (2001). Regional vascular selectivity of angiotensin II. *J Pharmacol Exp Ther* **297**, 736–345.

Jan MY, Hsiu H, Hsu TL, Wang WK & Wang Lin YY (2003). The physical conditions of different organs are reflected specifically in the pressure pulse spectrum of the peripheral artery. *J Cardiovas Eng* **3**, 21–29.

Kelly RP, Hayward CS, Avolio AP & O'Rourke MF (1989). Noninvasive determination of age-related changes in the human arterial pulse. *Circulation* **80**, 1652–1659.

Kizilova NN (2003). Reflection of pulse waves and resonance characteristics of arterial beds. *Fluid Dynamics* **38**, 772–781. Translated from (2003). *Izvestiya Rossiiskoi Academii Nauk Mekhanika Zhidkostii Gaza* **5,** 129–139.

Lappe RW & Brody MJ (1984). Mechanisms of the central pressor action of angiotensin II in conscious rats. *Am J Physiol* **246**, R56–R62.

Lin Wang YY, Jan MY, Shyu CS, Chiang CA & Wang WK (2004). The natural frequencies of the arterial system and their relation to the heart rate. *IEEE Trans Biomed Eng* **51**, 193–195.

Lu WA, Cheng CH, Lin Wang YY & Wang WK (1996). Pulse spectrum analysis of hospital patients with possible liver problems. *Am J Chin Med* **24**, 315–320.

Milnor WR (1989). Vascular impedance. In Hemodynamics, 2nd edn, ed. Collins N, pp. 188–189. Williams & Wilkins, Baltimore, MD, USA.

Murgo JP, Westerhof N, Giolma JP & Altobeli SA (1980). Aortic input impedance in normal man: relationship to pressure wave forms. *Circulation* **62**, 105–116.

Nicholes WW & Singh BM (2002). Augmentation index as a measure of peripheral vascular disease state. *Curr Opin Cardiol* **17**, 543–551.

O'Rourke MF, Pauca A & Jiang XJ (2001). Pulse wave analysis. *Br J Clin Pharmacol* **51**, 507–522.

Takazawa K, Tanaka N, Fujita M, Matsuka O, Saiki T, Aikawa M, Tamura S & Ibukiyama C (1998). Assessment of vasoactive agents and vascular aging by the second derivative of photoplethysmogram waveform. *Hypertension* **32**, 365–370.

Wang WK, Hsu TL, Bau JG & Wang-Lin YY (2003). Evaluation of herbal formulas by pulse analysis method. *Acta Pharmacol Sin* **24**, 145–151.

Wang WK, Hsu TL, Chiang Y & Wang YY (1997). Pulse spectrum study on the effect of sie-zie-tang and Radix aconiti. *Am J Chin Med* **25**, 357–366.

Wang WK, Hsu TL, Huang ZY & Wang YY(1995). Collective effect of a Chinese formula – a study of xiao-jian-zhong-tang. *Am J Chin Med* **23**, 299–304.

Wang WK, Lo YY, Chiang Y, Hsu TL & Wang Lin YY (1989). Resonance of organs with the heart. In Biomedical Engineering – An International Symposium, ed. Young WJ, pp. 259–268, Hemisphere, Washington, DC, USA.

Wang WK, Tsuei J, Chang HC, Hsu TL & Wang YY (1996). Pulse spectrum analysis of chemical factory workers with abnormal blood test. *Am J Chin Med* **24**, 199–203.

Wang YY, Chang SL, Wu YE, Hsu TL & Wang WK (1991). Resonance. The missing phenomenon in hemodynamics. *Circ Res* **69**, 246–249.

Wang YY, Lia WC, Hsiu H, Jan MY & Wang WK (2000). Effect of length on the fundamental resonance frequency of arterial models having radial dilatation. *IEEE Trans Biomed Eng* **47**, 1240–1241.

Wiener F, Morkin E, Skalak R & Fishman AP (1966). Wave propagation in the pulmonary circulation. *Circ Res* **XIX**, 834–850.

Young ST, Wang WK, Chang LS & Kuo TS (1989). Specific frequency properties of the renal and the superior mesenteric arterial beds in rats. *Cardiovas Res* **23**, 465–467.

Young ST, Wang WK, Chang LS & Kuo TS (1992). The filter properties of the arterial beds of organs in rats. *Acta Physiol Scand* **145**, 401–406.

Yu GL, Wang L & Wang WK (1994). Resonance in the kidney system of rats. *Am J Physiol* **36**, H1544–H1548.

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