# Analysis of short-term oscillations of R-R and arterial pressure in conscious dogs

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RIMOLDI, ORNELLA, SIMONA PIERINI, ANTONELLA FERRARI, SERGIO CERUTTI, MASSIMO PAGANI, AND ALBERTO MALLIANI. *Analysis of short-term oscillations of R-R and arterial pressure in conscious dogs.* Am. J. Physiol. 258 (Heart Circ. Physiol. 27): H967–H976, 1990.—We studied the neural determinants of the second (i.e., high frequency, HF)- and third-order (i.e., low frequency, LF) spontaneous oscillations of heart period (R-R interval) and arterial pressure (AP) in conscious dogs, with the hypothesis that they might furnish quantitative markers of autonomic controlling activities. Spectral analysis of simultaneous R-R and AP variabilities quantified these oscillations that were also evaluated in units normalized by total power to focus on the balance of these two major components. At rest we observed a prevalent HF component (~0.25 Hz) in R-R and AP variabilities that was synchronous with respiration. This HF component of R-R variability disappeared after atropine infusion and can be considered a marker mostly of vagal activity. When baroreceptor unloading, obtained by moderate hypotension, increased sympathetic activity the LF component increased in R-R, systolic, and diastolic AP variabilities. This increase in LF was not present after ganglionic blockade or after chronic arterial baroreceptor denervation. After chronic bilateral stellectomy, hypotension was not accompanied by an increase in LF component of R-R variability, while LF component remained in AP variability. An increase in LF component of R-R and AP variabilities was observed during transient coronary artery occlusion.

spectral analysis; sympathetic activity; vagal activity; autonomic balance; sympathectomy; atropine; ganglionic blockade

STUDIES ON THE NEURAL CONTROL of the circulation in intact animals or humans are usually based on the assessment of the properties of the various reflexes and of their interaction i.e., they mainly relate to phasic changes in functions.

Relatively few attempts have addressed the problem of the tonic cardiovascular control in intact animals or humans, most likely because of a lack of techniques able to directly monitor the levels of sympathetic and vagal regulatory tones. The recent introduction of spectral techniques for studying beat-by-beat cardiovascular variabilities has provided what seems to be an important tool for enhancing our understanding of tonic cardiovascular regulation (2, 5, 8, 20, 31, 35, 37). Studies in humans (10, 11, 31, 35) and conscious dogs (19, 31) suggest that the two major components of short-term heart rate variability i.e., the high-frequency (HF, ~0.25 Hz) respiration-linked component and the low-frequency (LF, ~0.1 Hz) component, could provide quantitative markers of vagal and sympathetic activities, respectively, and of their dynamic balance.

However, whereas there is substantial agreement on the hypothesis of a link between vagal tonic activity and the HF component of heart rate variability (1, 2, 9, 11, 31, 35, 37), the LF component has been interpreted as a marker of both vagal and sympathetic activities (1–3, 35) or as an indicator mainly of sympathetic tone and of its changes (18, 26, 31). These different views are likely to depend not only on the use of different biological and mathematical models but also on the assessment of the power of spectral components in absolute or normalized units.

By applying a similar technique to arterial pressure fluctuations (1, 6, 10, 30, 31), the magnitude of the third-order pressure waves (24, 33, 34), corresponding to the LF component of heart rate variability (1, 6, 11, 30–32), appeared to increase in conditions of sympathetic activation (17, 30–32). In general, this approach in the frequency domain provided a practical tool to detect various states of autonomic regulation, modeled as a changing balance between sympathetic and vagal tonic activities.

In the present experiments we used several groups of conscious dogs as follows: *1*) in control conditions; *2*) after chronic bilateral stellectomy; *3*) after chronic denervation of the aortic arch and of the carotid sinuses; and *4*) after implantation of a coronary hydraulic occluder. Experimental procedures were carried out to study the effects of baroreceptor loading and unloading, as a tool for increasing vagal or sympathetic reflex activities before and after various denervations or pharmacological interventions. Moreover, we studied the effects of a reflex sympathetic excitation elicited by transient coronary occlusion (21, 25, 28, 29). As a final methodological point we assessed the power of spectral components both in normalized and absolute units.

The results of this study seem to reinforce the hypothesis that the relative power of the LF and HF components, evaluated in normalized units, provide markers of the sympathovagal balance modulating heart rate variability. Likewise the LF component of arterial pressure

 

variability seems to provide a marker of the interaction between sympathetic activity and the peripheral smooth muscle vascular tone.

## METHODS

*Surgical preparation.* Mongrel dogs ($n = 26$) of either sex (20–25 kg body wt) were used for the study. All animals were lightly anesthetized with thiopental sodium (30 mg/kg iv; Farmotal, Farmitalia, Italy). A Tygon catheter was inserted into the femoral artery and advanced to the abdominal aorta; it was then secured with a purse-string suture and tunneled to the interscapular region.

Some of these animals ($n = 20$), under sterile conditions, underwent a left thoracotomy in one of the following intercostal spaces: the fifth for "coronary artery implant" ($n = 8$), the fourth for "arterial baroreceptor denervation" ($n = 6$), and the third for "bilateral stellectomy" ($n = 6$). For these surgical procedures animals were anesthetized with thiopental sodium (Farmotal, 30 mg/kg iv) followed by continuous infusion of fentanyl citrate (0.02 $\mu g \cdot kg^{-1} \cdot min^{-1}$ iv) and droperidol (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ iv) (Leptofen, Carlo Erba, Italy). Skeletal muscle paralysis was obtained with intermittent doses of succinylcholine after which the animals were intubated and ventilated with a positive-pressure pump (Harvard, Boston, MA) using room air and additional $O_2$ whenever necessary.

*Coronary artery implant.* After the pericardial sac was opened, a small tract (5 mm) of either the left circumflex ($n = 7$) or the left anterior descending coronary artery ($n = 1$) was carefully dissected near the bifurcation, and a miniature hydraulic occluder (4 mm ID) was placed around it. The volume of saline necessary to fully inflate the occluder so as to occlude the artery was also determined. A solid-state pressure transducer (Konigsberg Instruments, Pasadena, CA) was implanted in the left ventricle through the apical dimple. Tygon catheters were inserted in the left atrial appendage and in the descending thoracic aorta, respectively. All catheters were tunneled subcutaneously and exteriorized in the interscapular region.

*Stellectomy.* This was a two-phase procedure. First, by way of the thoracotomy in the third intercostal space, the left stellate ganglion and its branches were isolated and excised. The wound was closed, and the animal was allowed 10–15 days for recovery. Subsequently, using a similar anesthesia and through a right thoracotomy in the third intercostal space, the right stellate ganglion and its branches were excised as well. A second 15-day recovery period was allowed before resuming the study. Complete denervation was verified during surgery by close examination of the excised specimen.

*Arterial baroreceptor denervation.* This was also a two-phase procedure aimed at abolishing, or drastically reducing, baroreceptor influences, while leaving intact cardiac efferent innervation. Thus, initially, by way of the left thoracotomy in the fourth space, the adventitia surrounding the aortic arch and its branches, where baroreceptor fibers are known to be located, was dissected carefully avoiding any visible damage to the vagi or to the sympathetic nerve branches. The surgical wound was closed, and the animals were allowed 7 days for recovery. Subsequently, during transient anesthesia with thiopental sodium (20 mg/kg iv) and through a longitudinal midcervical incision the carotid sinus nerves were isolated and cut. The effects of this procedure on heart rate-blood pressure relationships were tested after full recovery by verifying that nitroglycerin infusion (32 $\mu g \cdot kg^{-1} \cdot min^{-1}$), which in the intact animals reduces mean arterial pressure by 7 ± 2 mmHg and increases heart rate by 41 ± 7 beats/min (see RESULTS), induced a greater pressure fall (20 ± 4 mmHg) and only insignificant changes in heart period. This resulted in discarding 4 of the 10 animals that had been so prepared.

*Recorded variables.* The electrocardiogram (lead II) was obtained with subcutaneous silver electrodes and an alternating-current (AC) amplifier. Heart rate was monitored continuously with a cardiotachometer triggered by the R wave. Systemic arterial and left atrial pressures were measured with the implanted catheters using strain-gauge transducers (Statham Instruments, Oxnard, CA). Respiratory movements were monitored with a pneumatic belt connected to a pressure transducer (Statham). Left ventricle pressure was measured with the implanted miniature pressure gauge that was calibrated statically in vitro and dynamically in vivo, using the left atrial and aortic pressures. The time derivative of left ventricular pressure (LV $dP/dt$) was obtained with an operational amplifier with a frequency response of 0.5–700 Hz. A triangular signal with a known slope was used to calibrate the differentiator. Data were recorded on a multichannel FM-type recorder (Racal-Dana Instruments, Southampton, England) and played back on a direct-writing recorder (Gould 2800, Cleveland, OH).

*Protocol.* Experiments were performed 1–2 wk postoperatively at a time when the dogs were apparently well and had recovered from the operation, as judged by their normal behavior, body temperature, and hematocrit. Given the general hypothesis that sympathetic and vagal controlling activities are the major determinants of short-term spontaneous oscillations in heart period and arterial blood pressure, we planned experiments in various conditions characterized by different levels of sympathetic and vagal tonic activities. The sympathovagal balance was altered with the following interventions. Efferent sympathetic excitation was obtained with nitroglycerin infusion accompanied by moderate hypotension and therefore by baroreceptor unloading. Efferent vagal excitation was obtained with phenylephrine infusion and baroreceptor stimulation. The animals were also tested after chronic denervation of carotid sinuses and aortic arch. Vagal efferent activity was antagonized with muscarinic-receptor blockade, while chronic bilateral stellectomy was performed to abolish the influence of the sympathetic innervation directed to the heart, while leaving intact a large fraction of the sympathetic outflow innervating the vasculature. Finally, a reflex sympathetic excitation was also obtained with a transient nonhypotensive coronary occlusion.

In the case of sympathetic activation, we predicted a decrease in total variance of heart rate variability accom-

panied by an increase in the normalized power of LF component and a decrease of HF component; simultaneously, we expected that LF component of arterial pressure variability would increase. Whereas, in the case of vagal activation, we predicted an increase in total variance of heart rate variability accompanied by an increase in the normalized power of HF component and a decrease of LF component. The use of normalized units was adopted to facilitate the comparison of spectral components in the face of large changes in total power (31).

All dogs were acquainted with the laboratory and trained to lie unrestrained on the recording table. During the recording sessions external influences, such as ambient light or noise and changes in the experimental team that could excite the dogs, were kept to a minimum.

While the animals ($n$ = 26) were lying flat on the table, ECG, aortic pressure, and respiratory movements were recorded continuously for 20–30 min followed by an infusion of nitroglycerin ($n$ = 14; 32 $\mu g \cdot kg^{-1} \cdot min^{-1}$ iv) or of phenylephrine ($n$ = 5; 10 $\mu g \cdot kg^{1} \cdot min^{-1}$ iv). During these infusions, after 5–10 min were allowed for a stabilization of the hemodynamic conditions, a recording of 20 min was performed.

Pharmacological blockades were then tested on separate days. Muscarinic-receptor mediated effects were blocked with atropine (0.1 mg/kg iv). The efficacy of the blockade was verified by the lack of a further increase in heart rate after a booster dose (0.1 mg/kg iv) of atropine. This intervention, by abolishing vagal efferent control of heart rate, induces a shift toward sympathetic predominance, the magnitude of which is also likely to be affected by the concomitant change in arterial pressure (14). Ganglionic transmission was blocked with trimethaphan (Arfonad, Roche, Switzerland) infused intravenously (10 $\mu g \cdot kg^{-1} \cdot min^{-1}$). This intervention would eliminate any neurally mediated variability. Nonhypotensive transient coronary artery occlusion was performed by inflating with saline the precalibrated hydraulic occluder for ~120 s and then releasing it. In these experiments, left ventricle pressure and its time derivative were also recorded.

*Data analysis.* ECG, arterial pressure, and respiration were played back from the FM tape and digitized at 300 sample/s per channel on a DEC MNC 11/23 minicomputer. The basic principles of the software for data acquisition and analysis have been previously described (5, 8, 31). Stationary sections of data of appropriate length were selected and analyzed as outlined in Fig. 1. The series of $N$-consecutive R-R intervals, i.e., tachogram, is first calculated and saved in the computer memory. Then from sections of tachogram of 512 interval values, mean and variance were derived. Although this length of tachogram has been used throughout the study as the best compromise between the need for a large time series, to achieve greater accuracy in the computation, and the need to obtain stationary recordings, which would be easier for shorter time periods, shorter recordings were used in the case of coronary artery occlusion. Second, the computer program automatically calculates the model that provides, by minimizing the confidence areas of polar coordinates (7), the best statistical estimate and prints out the power and frequency of every spectral component that is presented in absolute units as well as in normalized form. The power of LF and HF components in normalized units (nu) is computed as

$$(P_{LF})_{nu} = \frac{P_{LF}}{\sigma^2 - P_0} \cdot 100 \text{ and } (P_{HF})_{nu} = \frac{P_{HF}}{\sigma^2 - P_0} \cdot 100$$

where $P_{LF}$ and $P_{HF}$ are the powers of the components expressed in milliseconds squared. $\sigma^2$ (i.e., total variance) and $P_0$ are also expressed in milliseconds squared. $P_0$ is

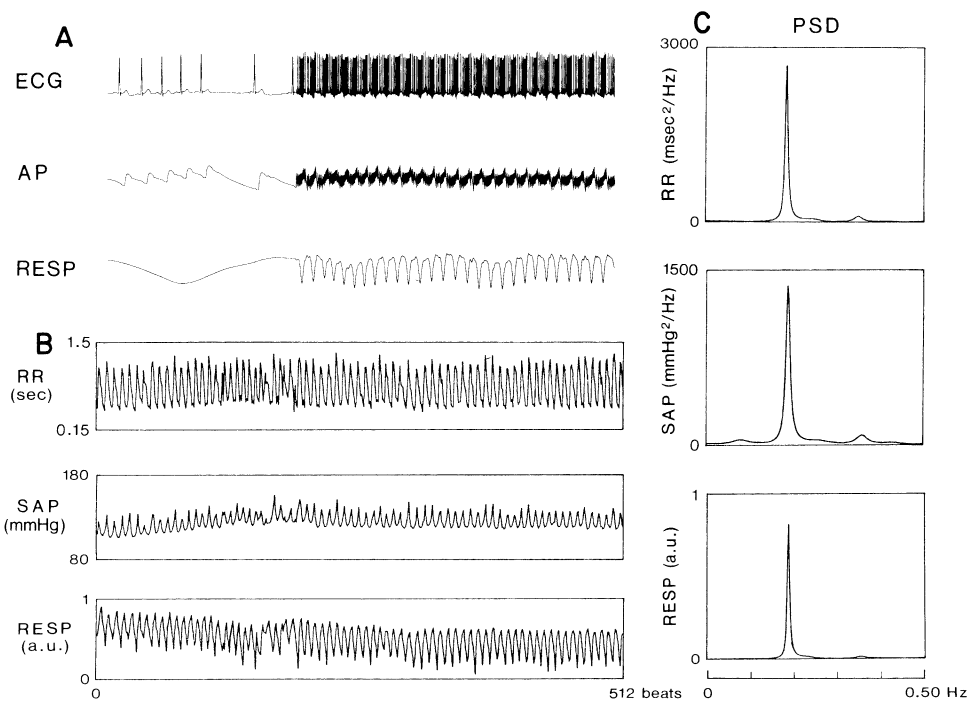


FIG. 1. Example of computer analysis of simultaneous analog recordings of electrocardiogram (ECG), arterial pressure (AP), and respiration (RESP) (*A*). After computer acquisition, synchronized tachogram (R-R), systogram (SAP), and respirogram (RESP) are obtained (*B*). From these data, autospectra (PSD) are computed (*C*) for, respectively, *top* to *bottom*, R-R, systolic arterial pressure (SAP), and respiration (RESP).

**TABLE 1.** *R-R interval and systolic and diastolic arterial pressure and their variabilities in conscious dogs at rest in intact conditions and after selective denervation procedures*

| | Mean Value | Variance | DC% | Low-Frequency Component | | | High-Frequency Component | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Spectral density | Normalized units | Frequency | Spectral density | Normalized units | Frequency |
| R-R | | | | | | | | | |
| Intact | 780±29 | 66,781±8,987 | 3±1 | 1,578±504 | 3±1 | 0.13±0.01 | 45,000±6,482 | 68±3 | 0.24±0.01 |
| Stellectomy | 765±13 | 44,109±10,373* | 3±1 | 864±668 | 4±3 | 0.09±0.06* | 27,226±7,488 | 61±5 | 0.24±0.01 |
| Atropine | 353±22* | 36±17 | 5±5 | 28±16 | 79±3* | 0.04±0.01 | 3±1 | 15±4* | 0.23±0.01* |
| TABD | 578±65* | 12,623±8,002 | 11±3 | 20±20 | 0±0* | Unmeasurable | 8,952±5,722 | 81±4* | 0.18±0.01* |
| SAP | | | | | | | | | |
| Intact | 119±2 | 69±15 | 21±4 | 7±1 | 16±3 | 0.09±0.01 | 29±4 | 62±3 | 0.24±0.01 |
| Stellectomy | 135±8* | 61±24 | 34±10* | 3±2 | 9±3* | 0.11±0.01* | 27±12* | 73±4* | 0.25±0.01 |
| Atropine | 129±6 | 8±2 | 0±0* | 5±1 | 65±3* | 0.04±0.01* | 2±1 | 20±3* | 0.27±0.03* |
| TABD | 145±12* | 59±23 | 58±13* | 0.1±0.1* | 1±1* | Unmeasurable | 16±6 | 81±6* | 0.18±0.02* |
| DAP | | | | | | | | | |
| Intact | 71±2 | 109±24 | 10±2 | 10±4 | 10±2 | 0.09±0.01 | 68±15 | 69±2 | 0.24±0.01 |
| Stellectomy | 76±2* | 75±18 | 22±7* | 1±1 | 7±4* | 0.08±0.01 | 36±14 | 64±6* | 0.24±0.01 |
| Atropine | 92±5* | 6±2 | 6±7* | 4±2 | 73±5* | 0.04±0.01 | 1±0.3 | 17±4* | 0.27±0.04* |
| TABD | 104±10* | 24±9 | 51±11* | 1±0.4 | 11±4* | 0.06±0.01 | 6±2 | 68±8* | 0.18±0.01* |

Intact, $n = 26$; stellectomy, $n = 6$; atropine, $n = 6$; TABD, $n = 6$. Mean value of R-R is ms; and variance and spectral density of R-R in $ms^2$. Mean value of SAP and DAP in mmHg, and variance and spectral density of SAP and DAP in $mmHg^2$. Frequency in Hz. R-R, R-R interval; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; TABD, total arterial baroreceptor denervation; and DC%, fractional power of direct-current component of power spectral density; see text for details. * Significant contrast $P < 0.05$.

the power of the very low-frequency component, with a period near 0 Hz (and <0.03 Hz), that cannot be properly analyzed with the methodology of this study. Recent reports, employing a different methodology, suggest that the analysis of long periods of uninterrupted data can provide a fruitful approach to the study of the very low frequency phenomena (36). However, because these oscillations, at the moment, cannot be interpreted from a biological point of view, they are disregarded in analysis, but indicated in Tables 1–4 as the percent direct current (DC%), for numerical reference. As already reported (31), only components >5% of total power were considered significant. Furthermore, because in individual animals smaller components could also be present, the sum of the LF and HF component represented only ~70–85% of total variability after subtraction of the DC component.

The use of normalization procedure becomes important in facilitating the comparison between spectra that show pronounced differences in total power (i.e., variance) or in the DC% component (12, 31); accordingly, in the text, spectral powers are expressed in normalized units, unless otherwise specified. As an example, muscarinic blockade reduces the total power of R-R variability by about three orders of magnitude, hence, the absolute power of both the LF and HF components appears drastically reduced (if expressed in milliseconds squared); however, their balance is shifted to a predominance of the LF components, as shown both by absolute and normalized units. Obviously in individual animals and various instances the power of the LF and HF spectral components depend on a complex interplay of neural circuits regulating sympathetic and vagal tonic activities and on the target function responsiveness, which at the moment cannot be precisely assessed. Hence, spectral components and simplified neural models can furnish only a crude interpretation of the complexity of the dynamic balance between the sympathetic and vagal activities regulating heart period and arterial pressure.

It should also be mentioned that with the method of analysis employed in this study the periodicity of the phenomena is measured as a function of cardiac beats, i.e., cycles per beat. This, however, can be converted into Hertz equivalents (henceforth indicated by Hz) by dividing it by the average R-R interval length. To compute the spectrum of both systolic and diastolic arterial pressure a similar procedure is used. Spectral analysis of the respiratory signal was performed on the signal sampled once every cardiac cycle.

*Statistics.* The results are expressed as means ± SE. Differences were assessed by analysis of variance, using the Scheffé and Bonferroni tests for the multiple comparisons. Whenever indicated, the significance of the changes from control produced by hemodynamic interventions was also assessed with the $t$ test for paired observation. Differences were considered significant with a $P$ value $< 0.05$ (4). Stepwise regression analysis was used to assess the relative interdependance of various spectral indexes.

## RESULTS

*Intact animals.* While the animals ($n = 26$) were lying quietly on the table, their heart rate (HR) was 79 ± 3

TABLE 2. *Changes in R-R interval and systolic and diastolic arterial pressure and their variabilities, produced by intravenous nitroglycerin in conscious dogs*

| | Mean Value | Variance | DC% | Low-Frequency Component | | | High-Frequency Component | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Spectral density | Normalized units | Frequency | Spectral density | Normalized units | Frequency |
| R-R | | | | | | | | | |
| Intact | −254±43 | −56,660±11,227 | 13±5 | 417±1,300 | 31±5 | 0.03±0.02 | −41,163±9,636 | −23±6 | 0.02±0.03 |
| Stellectomy | −158±25 | −41,740±9,868 | −3±2 | −854±670 | −3±3 | −0.02±0.03 | −25,688±7,083 | 16±8 | 0.0±0.02 |
| Atropine | −27±29 | 107±101 | 0±0 | 20±17 | 7±5 | 0.01±0.01 | −1±1 | −4±2 | Unmeasurable |
| TABD | −48±24 | −2,248±1,175 | 2±2 | 60±67 | 2±3 | 0.03±0.04 | −1,610±1,016 | 2±4 | 0.03±0.02 |
| SAP | | | | | | | | | |
| Intact | −19±3‡ | −39±23 | −4±2 | 9±4‡ | 41±5 | −0.03±0.01 | −14±4† | −34±5‡ | 0.05±0.03 |
| Stellectomy | −25±5‡ | −46±22 | 3±15 | 1±1 | 30±6 | −0.01±0.02 | −23±10 | −28±9‡ | 0.0±0.01 |
| Atropine | −15±13 | 10±14 | 0±0 | 11±10 | 11±12 | 0.01±0.01 | 0.02±1 | −11±8 | 0.03±0.07 |
| TABD | −27±8‡ | −36±22 | −11±12 | 2±1 | 13±8 | 0.02±0.01 | −9±7 | −18±5‡ | 0.01±0.01 |
| DAP | | | | | | | | | |
| Intact | −4±3 | −72±33 | 11±9 | −3±8 | 48±6 | 0.0±0.02 | −52±22‡ | −40±4 | 0.02±0.03 |
| Stellectomy | −3±2 | −65±15† | 14±12 | 2±1 | 36±4 | 0.04±0.02 | −33±13 | −25±4 | 0.01±0.02 |
| Atropine | −16±11 | 10±8 | −6±6 | 8±7 | 11±10 | 0.01±0.01 | −1±1 | −9±3 | 0.1±0.1 |
| Tabd | −17±4‡ | −9±13 | 5±12 | 1±1 | 18±11 | 0.02±0.03 | −4±2 | −22±6 | 0.01±0.02 |

Intact, $n = 14$; stellectomy, $n = 6$; atropine, $n = 6$; TABD, $n = 6$. SAP, systolic arterial pressure; DAP, diastolic arterial pressure; TABD, total arterial baroreceptor denervation; DC%, fractional power of direct-current component of the power spectral density. Mean value of R-R in ms, and variance and spectral density of R-R in $ms^2$. Mean value of SAP and DAP in mmHg, and variance and special density of SAP and DAP in $mmHg^2$. Frequency in Hz. * Significant contrast $P < 0.05$. † Significant difference from paired control $P < 0.01$. ‡ Significant difference from paired control $P < 0.05$.

beats/min, and their mean arterial pressure (MAP) was 85 ± 2 mmHg. Under these conditions, the R-R period, the systolic and diastolic arterial pressure (SAP and DAP, respectively) values underwent beat-by-beat variations around their mean values. Such oscillations are shown in Fig. 1 for R-R interval and SAP, together with respiration.

Spectral analysis can demonstrate that the major fraction of these variations is not random but possesses definite rhythms (Table 1). Spectral components are presented both in absolute and normalized units; the latter to account for the possible large differences in total power, i.e., variance, characterizing individual autospectra (31). The most powerful one of these is synchronous with respiration and occurs in the case of Fig. 1 at ~0.19 Hz as shown by the power spectral density (PSD) of all three variables displayed. However, in Fig. 1 a smaller component is evident in the LF range of the spectrum of SAP variability and occurs at a frequency of ~0.08 Hz. Such a LF component was detectable in all spectral analysis of SAP and DAP variabilities, whereas in the case of R-R variability it was observed only in 12 of 26 animals (Table 1). In the present data, LF had a frequency range of 0.04–0.16 Hz, and HF had a range of 0.16–0.4 Hz.

*Nitroglycerin infusion.* This infusion ($n = 14$) reduced MAP by 7 ± 2 mmHg and raised HR by 41 ± 7 beats/min. As already reported (31), this intervention was used to induce a sympathetic activation.

During the steady state, R-R and its variance were significantly reduced from the values present in control conditions (Table 2). Furthermore, the spectra were altered as a LF component of a magnitude (35 ± 5 nu) similar to the HF oscillation (43 ± 5 nu) was present in all animals studied (Fig. 2). Under these conditions, in which two major spectral components were simultaneously present, stepwise regression analysis indicated an

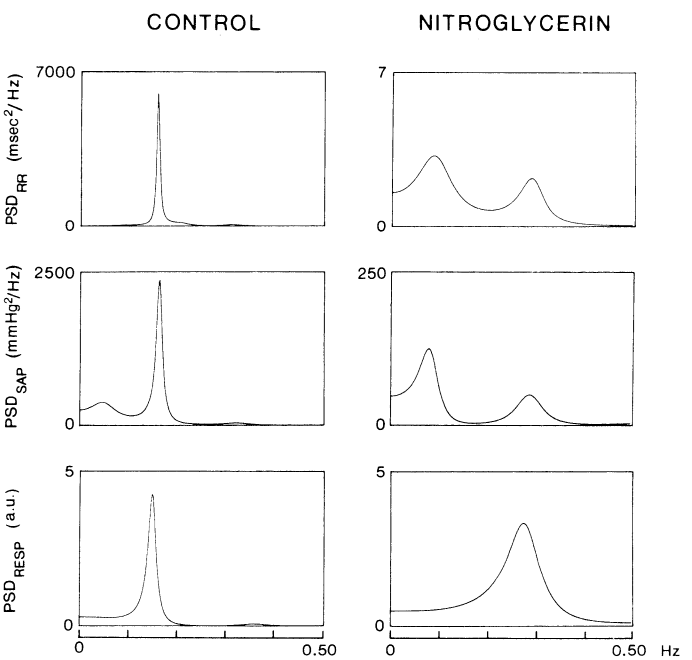


FIG. 2. Effects of 32 $\mu g \cdot kg^{-1} \cdot min^{-1}$ iv nitroglycerin on R-R and systolic arterial pressure (SAP) variabilities and on respiration (RESP). Note marked predominance of respiratory component in both R-R and SAP autospectra ($PSD_{RR}$ and $PSD_{SAP}$) at control, and note appearance of a large low-frequency component during nitroglycerin infusion.

TABLE 3. *R-R interval and systolic and diastolic arterial pressure and their variabilities at control and during intravenous phenylephrine infusion in conscious dogs (n = 5)*

| | Mean Value | Variance | DC% | Low-Frequency Component | | | High-Frequency Component | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Spectral density | Normalized units | Frequency | Spectral density | Normalized units | Frequency |
| R-R | | | | | | | | | |
| Control | 722±49 | 53,159±18,207 | 7±4 | 1,954±802 | 5±2 | 0.11±0.01 | 34,044±10,617 | 67±8 | 0.22±0.02 |
| Phenylephrine | 1,017±100* | 210,734±43,640* | 2±1 | 0±0* | 0±0* | Unmeasurable* | 153,384±37,961* | 73±6 | 0.24±0.03 |
| SAP | | | | | | | | | |
| Control | 132±7 | 84±18 | 21±6 | 6±1 | 10±2 | 0.10±0.01 | 41±10 | 63±4 | 0.23±0.01 |
| Phenylephrine | 178±3* | 229±62* | 20±6 | 10±3 | 9±3 | 0.08±0.01 | 106±35 | 60±6 | 0.22±0.03 |
| DAP | | | | | | | | | |
| Control | 63±2 | 97±39 | 9±4 | 5±1 | 8±2 | 0.10±0.01 | 58±24 | 66±4 | 0.23±0.01 |
| Phenylephrine | 95±5* | 284±84* | 10±1 | 10±4 | 4±2 | 0.06±0.01 | 151±42* | 61±10 | 0.23±0.04 |

Mean value of R-R in ms, and variance and spectral density of R-R in $ms^2$. Mean value of SAP and DAP in mmHg, and variance and spectral density of SAP and DAP in $mmHg^2$. Frequency in Hz. SAP, systolic arterial pressure; DAP, diastolic arterial pressure; DC%, fractional power of direct-current component of power spectral density. * Significant difference $P < 0.05$.

interdependence between them when assessed both in normalized ($r = 0.40$) and absolute ($r = 0.47$) units.

Likewise, both SAP and DAP and their variances were reduced during the infusion (Table 2), and in their spectra a significant increase in the normalized power of the LF component was observed. The HF component, on the other hand, was reduced from the control value both in absolute and normalized units. Respiratory frequency was higher during nitroglycerin infusion (Fig. 2).

*Phenylephrine infusion.* This infusion ($n = 5$) raised MAP by 29 ± 6% and decreased heart rate by 28 ± 3%. Under such conditions the variance values of R-R, SAP, and DAP augmented significantly (Table 3). The spectra of SAP and DAP were not modified by the infusion, as the power of both the LF and HF component, expressed in normalized units, were similar to those observed during control recordings. On the other hand, the small LF component present in control conditions in the spectrum of R-R variability was no longer discernible during phenylephrine infusion (Table 3).

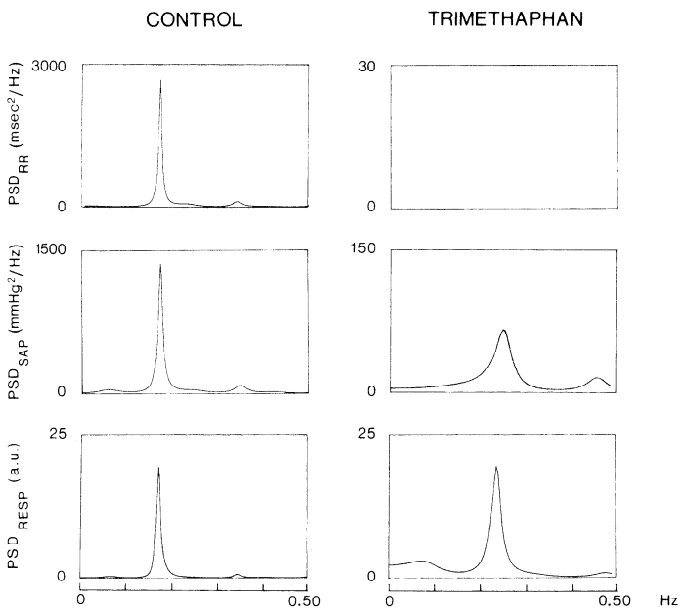


FIG. 3. Effects of trimethaphan infusion (10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ iv) on R-R and systolic arterial pressure (SAP) autospectra ($PSD_{RR}$ and $PSD_{SAP}$) and on respiration ($PSD_{RESP}$) in a conscious dog. Note that during infusion, which blocks ganglionic transmission (*right*), R-R variability disappears, while a sizable respiratory component is still present in $PSD_{SAP}$.

*Ganglionic blockade.* Experiments ($n = 5$) with trimethaphan infusion were used to assess the effects of the blockade of ganglionic transmission. This infusion reduced MAP drastically (52 ± 7%) and was accompanied by a consistent tachycardia (54 ± 15%). R-R variance was almost totally abolished (−99 ± 0.2%), and consequently no spectral components were discernible. However, a small respiratory oscillation was present in the spectra of both SAP (56 ± 6 nu) and DAP (59 ± 7 nu) variabilities (Fig. 3). Their variance values (9 ± 3 and 4 ± 1 $mmHg^2$, respectively) were significantly smaller than in control conditions.

*Stellectomy.* The role played by sympathetic cardiac innervation was assessed in dogs ($n = 6$) that underwent chronic bilateral stellectomy. As already described (31), this procedure did not modify the base-line values of heart rate and arterial pressure nor the variabilities of R-R, SAP, and DAP (Table 1, Fig. 4). The changes in MAP and heart rate produced by nitroglycerin infusion were similar to those observed in intact animals. However, spectral analysis indicated that during nitroglycerin hypotension a LF component could be detected only in the SAP (Fig. 4) and DAP spectra (Table 2), whereas it was no longer present in the R-R variability spectrum. The normalized power of the HF component of heart rate variability during hypotension was greater in denervated animals.

*Muscarinic blockade.* Intravenous atropine ($n = 6$) was used to block pharmacologically vagal efferent activity. This intervention raised heart rate substantially (115 ± 31%), while increasing MAP only slightly (9 ± 3 mmHg). During muscarinic blockade, R-R, SAP, and DAP variances were reduced and the spectra of all three variabilities were largely modified; LF and HF components were both reduced in absolute units, and their balance was shifted to a predominant LF both in absolute and normalized units. Furthermore, LF center frequency tended to values lower than in control conditions (Table 1). The

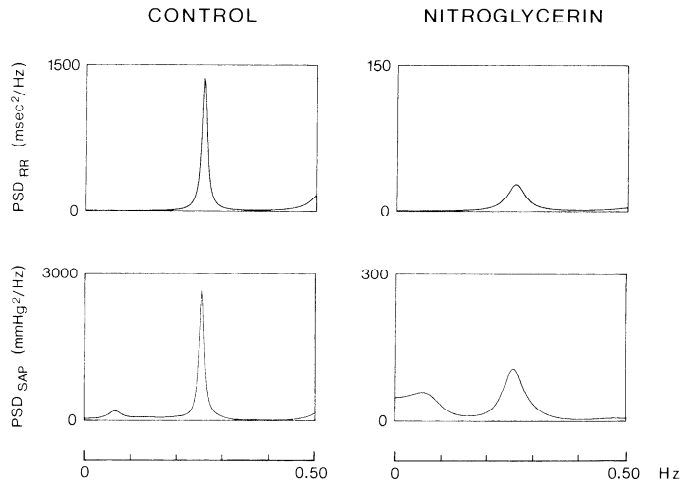


FIG. 4. Effects of nitroglycerin infusion (32 $\mu g \cdot kg^{-1} \cdot min^{-1}$ iv) in a conscious dog, after recovery from bilateral stellectomy, which abolishes a large fraction of cardiac sympathetic innervation. Note that, at control, autospectra (PSD) of both R-R and systolic arterial pressure (SAP) variabilities demonstrate a largely predominant respiratory component, much the same as in intact animals. In this case, however, during nitroglycerin infusion, owing to selective cardiac sympathetic denervation, a low-frequency component is seen only in $PSD_{SAP}$ and not in $PSD_{RR}$.

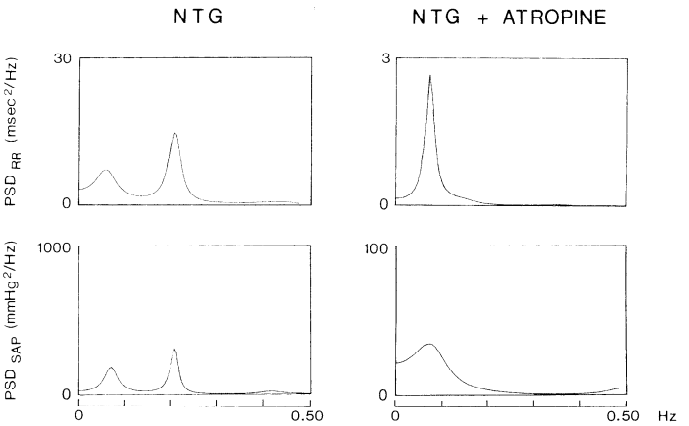


FIG. 5. Changes produced by muscarinic blockade (atropine 0.1 $\mu g$/kg iv) on autospectra (PSD) of R-R and systolic arterial pressure (SAP) variabilities during nitroglycerin (NTG) hypotension. Note disappearance of high-frequency respiratory components in both $PSD_{RR}$ and $PSD_{SAP}$, as well as maintenance of a predominant low-frequency component, in the face of a reduced total variability.

combined effects of nitroglycerin hypotension and atropine administration consisted of a slight further increase of LF for R-R, SAP, and DAP (Table 2) compared with atropine alone; this change was directionally similar both in absolute and normalized units.

Figure 5 shows the near abolition of the respiratory component and the evidence of a residual power concentrated in a predominant LF component, as a consequence of atropine administration during nitroglycerin infusion.

*Arterial baroreceptor denervation.* After this procedure, the animals ($n = 6$) demonstrated a higher heart rate (109 ± 9 beats/min) and MAP (117 ± 8 mmHg) compared with the neurally intact condition. Thus R-R was smaller, and SAP and DAP were significantly higher (Table 1). All variance values were reduced. With regards to the spectral analysis, only a single respiratory component was present in R-R, SAP, and DAP variabilities (Fig. 6 and Table 1).

Nitroglycerin infusion, reduced MAP (−20 ± 4 mmHg) more ($P < 0.001$) than in intact animals, without changing heart period significantly as a reflection of the surgical abolition of a large fraction of baroreceptor afferents (see METHODS). During the nitroglycerin hypotension, at variance with the intact animals, there was only a minor and not significant increase from control of the LF component of R-R, SAP, and DAP variabilities (Fig. 6 and Table 2). It should be noticed that under these conditions the absolute power of the HF component of SAP and DAP variabilities decreased from the value observed before the infusion.

*Brief coronary artery occlusion.* During the occlusion of a distal branch of either the left circumflex or anterior descending coronary artery, maintained for ~120 s, the animals ($n = 8$) did not display behavioral signs attributable to pain. Mean arterial pressure was not significantly modified (1 ± 2 mmHg), whereas heart rate increased by 51 ± 9%. R-R and its variance were reduced significantly (Table 4). Figure 7 illustrates the marked and parallel changes in the spectra of R-R and SAP variabilities, which were both characterized during the occlusion by an increase in the normalized power of the LF component (Table 4).

## DISCUSSION

*Spectral analysis of heart rate variability and neural control of heart rate.* This study confirms that in the conscious dog in control resting conditions, there is a single major component in the spectrum of heart rate variability that is synchronous with respiration. This

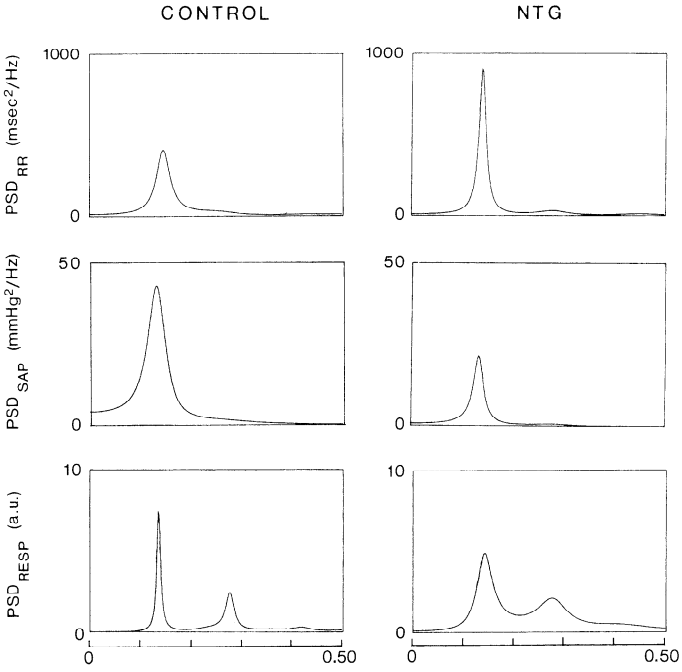


FIG. 6. Effects of hypotension produced by nitroglycerin (NTG) infusion (32 $\mu g \cdot kg^{-1} \cdot min^{-1}$ iv) on $PSD_{RR}$ and $PSD_{SAP}$ and on respiration (RESP) in a conscious dog after full recovery from arterial baroreceptor denervation. Note that both at control and during (NTG) infusion there is only one major respiratory component for both $PSD_{RR}$ and $PSD_{SAP}$.

TABLE 4. *R-R interval and systolic and diastolic arterial pressure and their variabilities in conscious dogs at rest and during transient coronary artery occlusion (n = 8)*

| | Mean Value | Variance | DC% | Low-Frequency Component | | | High-Frequency Component | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Spectral density | Normalized units | Frequency | Spectral density | Normalized units | Frequency |
| R-R | | | | | | | | | |
| Control | 710±40 | 44,659±10,655 | 3±1 | 831±412 | 3±1 | 0.14±0.02 | 29,773±7,890 | 74±4 | 0.28±0.04 |
| CAO | 474±22* | 3,061±1,511* | 15±15 | 716±342 | 47±10* | 0.09±0.02 | 672±265* | 42±9* | 0.31±0.04 |
| SAP | | | | | | | | | |
| Control | 114±5 | 39±14 | 11±4 | 6±2 | 17±3 | 0.07±0.01 | 24±10 | 62±5 | 0.27±0.04 |
| CAO | 108±4* | 14±4 | 17±9 | 6±2 | 52±8* | 0.08±0.01 | 3±2 | 37±7* | 0.35±0.05* |
| DAP | | | | | | | | | |
| Control | 74±4 | 77±29 | 8±4 | 4±2 | 9±2 | 0.07±0.02 | 55±22 | 69±5 | 0.27±0.04 |
| CAO | 77±3 | 25±8 | 15±8 | 7±2 | 56±7* | 0.04±0.01 | 3±1* | 34±5* | 0.35±0.05* |

SAP, systolic arterial pressure; DAP, diastolic arterial pressure; CAO, transient (~120 s) coronary artery occlusion; DC%, fractional power of direct-current component of power spectral density. Mean value for R-R in ms, and variance and spectral density in $ms^2$. Mean value for SAP and DAP in mmHg, and variance and spectral density for SAP and DAP in $mmHg^2$. Frequency in Hz. * Significant difference $P < 0.05$.

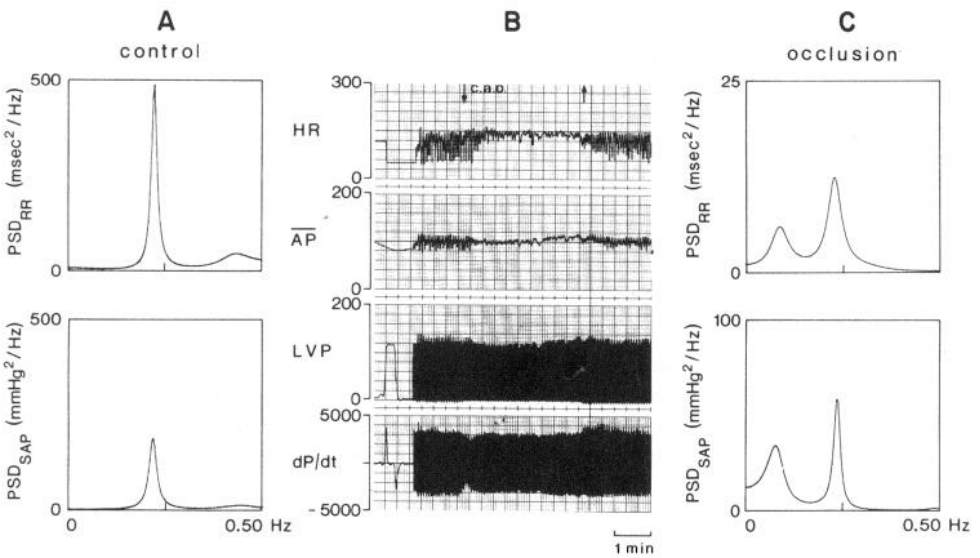


FIG. 7. Effects of a transient, nonhypotensive, coronary artery occlusion (arrows, *B*) on autospectra of R-R and systemic arterial pressure (SAP) variabilities (PSD) in a conscious dog. Note that during occlusion (*C*) there is a reduction in total variance from control (*A*), together with appearance of a large low-frequency component in both R-R and SAP PSD, as a reflection of increase in sympathetic efferent activity.

might reflect the dominant role of respiration (19) on R-R variability as suggested, also, by the striking similarity between the autospectrum of R-R variability and that of the respiratory wave form. As to the lower end of the spectrum, we observed a LF component, at ~0.1 Hz, of very small amplitude only in about half of the animals.

An important determinant of the amplitude of the respiratory component of R-R variability, i.e., respiratory arrhythmia, is vagal efferent activity (1, 2, 9, 15, 16, 23, 35), as indicated by the drastic reduction of the HF component expressed both in absolute and normalized units, with vagotomy or muscarinic blockade.

Furthermore, the reduction of total variability with muscarinic blockade (1, 2, 35) and its increase during a rise in arterial pressure induced by phenylephrine, an experimental maneuver that reflexly increases vagal efferent activity, suggest that the vagal outflow also affects R-R variance. Thus both the normalized power of the respiratory spectral component and total R-R variability, i.e., variance, seem to reflect vagal efferent control.

As to sympathetic activity and its changes, we have already suggested that it could be assessed by the normalized power of the LF component (18, 26, 27, 30), whereas others have indicated that both vagal and sympathetic activities (1–3, 35) determine the absolute power of LF component, expressed in milliseconds squared. To reconcile this potential contradiction, in the present study we planned to investigate the role of sympathetic activity in determining the power of the LF component expressed both in normalized units and in milliseconds squared. In this context, HF and LF components should not be viewed as specific measures of vagal and sympathetic neural activity but as markers of their dynamic balance. Experimental results were directionally similar; however, they were more consistent when expressed in normalized units to account for individual changes. Concerning this methodological problem, a crucial example is furnished by the effects of atropine administration that reduced total variance by more than 99%; yet in these conditions the residual power was largely concentrated in the LF component measured both in absolute and normalized units. This seems to provide a further support to the link between sympathetic modulation and LF component (31).

We also assessed the effects of various denervation procedures on the changes produced by moderate hypotension on heart rate variability. After bilateral stellectomy the disappearance of the increase in the LF com-

ponent of R-R variability induced in intact animals by nitroglycerin infusion indicates a direct role of cardiac sympathetic nerves (31) in determining this component. As to the effects of nitroglycerin hypotension, it should be further specified that in intact animals this maneuver reduced total variance, while LF was increased and HF decreased, thus indicating a change in their balance; after stellectomy the same intervention still reduced total variance but did not induce any consistent change in spectral components of R-R variability probably as a consequence of the interruption of the neural outflow mediating the LF increase in the intact animal.

In baroreceptor-denervated animals, nitroglycerin infusion was not accompanied by an increase in the LF component in accordance with the major role attributed to acute baroreceptor unloading as a mechanism for altering the sympathovagal balance in favor of sympathetic predominance.

The observation that during transient coronary artery occlusion there was a marked increase, in normalized units, of the LF component and a reduction of the HF component, concomitant with a drastic diminution of total variability, indicates the existence of a sympathetic excitatory reflex originating from the heart (25, 28, 29) and that the changes in spectral components are adequate to signal its occurrence. It is important to consider that during this intervention the role of baroreceptor mechanisms in inducing a sympathetic activation was likely to be minimal, because mean arterial pressure did not change, and that only a minor reduction in systolic and increase in diastolic pressures were observed. Furthermore, the increase in the LF component with transient myocardial ischemia was even more evident than that observed with baroreceptor unloading.

Finally, we should also point out that with muscarinic blockade and during transient ischemia the center frequency of the LF component tends toward values lower than in control, as observed in humans recovering from acute myocardial ischemia (26); this finding, while difficult to interpret at the moment, further indicates the potential advantage of using a technique that allows the measurement of the frequency of the individual spectral components.

*Arterial pressure variability and sympathetic vascular control.* Spectral analysis of systolic and diastolic arterial pressure variabilities also demonstrated, at rest, one major component synchronous with respiration.

This second-order (24) oscillation was likely to reflect mainly the direct mechanical effects of breathing (13) on intrathoracic blood vessels and stroke volume, although the beat-by-beat fluctuations of the latter are likely to depend also on the beat-by-beat changes in R-R (11) that are largely neurally mediated.

The observation that during ganglionic blockade that totally abolishes R-R variability, the respiratory component of arterial pressure variability was still present, albeit reduced in amplitude, indicates that both neural and mechanical factors are important determinants of this component. As to the LF component of arterial pressure variability that corresponds to third-order oscillations (24, 33, 34), the data of this study support the hypothesis that the sympathetic nervous system plays an important role in determining their amplitude. Accordingly, this amplitude may furnish a convenient marker of sympathetic vascular control.

The proposed relation between the LF component of arterial pressure variability and sympathetic activity is also in keeping with the findings that ganglionic-transmission blockade, abolishing the possibility of a sympathetic activation impinging on blood vessels, was associated with a marked hypotension in absence of a LF component in arterial pressure. In contrast, when a more moderate hypotension, as that obtained with nitroglycerin infusion, was produced in animals with functional sympathetic innervation, a marked increase in the fractional power of the LF component was observed.

It should be emphasized that bilateral stellectomy, which leaves intact vascular innervation, did not abolish the LF component of arterial pressure variability both in control conditions and during nitroglycerin hypotension, as already reported (31).

Finally, a marked increase in the LF component of arterial pressure was also present during transient coronary occlusion, suggesting that the excitatory sympathetic reflex initiated by the occlusion (21, 22, 25, 28, 29) has the peripheral vasculature amongst its functional targets.

In conclusion, the assessment by spectral techniques of second- and third-order cardiovascular rhythms, with the physiological hypothesis that their reciprocal changes might reflect a similar reciprocal organization existing between, respectively, vagal and sympathetic activities regulating cardiovascular function, seems to provide valuable markers of the dynamic sympathovagal balance.

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Received 5 December 1988; accepted in final form 19 October 1989.

REFERENCES

1. AKSELROD, S., D. GORDON, J. B. MADWEED, N. C. SNIDMAN, D. C. SHANNON, AND R. J. COHEN. Hemodynamic regulation: investigation by spectral analysis. *Am. J. Physiol.* 249 (*Heart Circ. Physiol.* 18): H867–H875, 1985.
2. AKSELROD, S., D. GORDON, F. A. UBEL, D. C. SHANNON, A. C. BARGER, AND R. J. COHEN. Power spectrum analysis of heart rate fluctuations: a quantitative probe of beat-to-beat cardiovascular control. *Science Wash. DC* 213: 220–222, 1981.
3. ARAI, Y., P. PAUL, P. ALBRECHT, L. H. HARTLEY, L. S. LILLY, R. J. COHEN, AND W. S. COLUCCI. Modulation of cardiac autonomic activity during and immediately after exercise. *Am. J. Physiol.* 256 (*Heart Circ. Physiol.* 25): H132–H141, 1989.
4. ARMITAGE, P. *Statistical Methods in Medical Research.* Oxford, UK: Blackwell Scientific, 1971.
5. BARTOLI, F., G. BASELLI, AND S. CERUTTI. Application of identification and linear filtering algorithms to the R-R interval measurements. In: *Computers in Cardiology.* Silver Spring, MD: IEEE Computer Soc., 1982, p. 485–488.
6. BASELLI, G., S. CERUTTI, S. CIVARDI, D. LIBERATI, F. LOMBARDI, A. MALLIANI, AND M. PAGANI. Spectral and cross-spectral analysis of heart rate and arterial blood pressure variability signals. *Comput. Biomed. Res.* 19: 520–534, 1986.
7. BASELLI, G., S. CERUTTI, S. CIVARDI, F. LOMBARDI, A. MALLIANI, M. MERRI, M. PAGANI, AND G. RIZZO. Heart rate variability signal processing: a quantitative approach as an aid to diagnosis in cardiovascular pathologies. *Int. J. Bio-Med. Comput.* 20: 51–70, 1987.

8. BROVELLI, M., G. BASELLI, S. CERUTTI, S. GUZZETTI, D. LIBERATI, F. LOMBARDI, A. MALLIANI, M. PAGANI, AND P. PIZZINELLI. Computerized analysis for an experimental validation of neurophysiological models of heart rate control. In: *Computers in Cardiology*. Silver Spring, MD: IEEE Computer Soc. 1983, p. 205–208.
9. CHESS, G. F., R. M. K. TAM, AND F. R. CALARESU. Influence of cardiac neural inputs on rhythmic variations of heart period in the cat. *Am. J. Physiol.* 228: 775–780, 1975.
10. DE BOER, R. W., J. M. KAREMAKER, AND J. STRACKEE. Relations between short term blood-pressure fluctuations and heart rate variability in resting subjects: I. a spectral analysis approach. *Med. Biol. Eng. Comput.* 23: 352–358, 1985.
11. DE BOER, R. W., J. M. KAREMAKER, AND J. STRACKEE. Hemodynamic fluctuations and baroreflex sensitivity in humans: a beat-to-beat model. *Am. J. Physiol.* 253 (*Heart Circ. Physiol.* 22): H680–H689, 1987.
12. DE TRAFFORD, J. C., K. LAFFERTY, R. I. KITNEY, L. T. COTTON, AND V. C. ROBERTS. Modelling of the human vasomotor control system and its application to the investigation of arterial disease. *IEEE Proc.* 129: 646–650, 1987.
13. DORNHORST, A. C., P. HOWARD, AND G. L. LEATHART. Respiratory variations in blood pressure. *Circulation* 6: 553–558, 1952.
14. ECKBERG, D. L., R. F. REA, O. K. ANDERSON, T. HEDNER, J. PERNOW, J. M. LUNDBERG, AND B. G. WALLIN. Baroreflex modulation of sympathetic activity and sympathetic neurotransmitters in humans. *Acta Physiol. Scand.* 133: 221–231, 1988.
15. FALLEN, E. L., M. V. KAMATH, D. N. GHISTA, AND D. FITCHETT. Spectral analysis of heart rate variability following human heart transplantation: evidence for functional reinnervation. *J. Auton. Nerv. Syst.* 23: 199–206, 1988.
16. FOUAD, F. M., R. C. TARAZI, C. M. FERRARIO, S. FIGHALY, AND C. ALICANDRI. Assessment of parasympathetic control of heart rate by a noninvasive method. *Am. J. Physiol.* 246 (*Heart Circ. Physiol.* 15): H838–H842, 1984.
17. FURLAN, R., S. DELL'ORTO, W., CRIVELLARO, P. PIZZINELLI, S. CERUTTI, F. LOMBARDI, M. PAGANI, AND A. MALLIANI. Effects of tilt and treadmill exercise on short-term variability in systolic arterial pressure in hypertensive man. *J. Hypertens.* 5: S423–S425, 1987.
18. GUZZETTI, S., E. PICCALUGA, R. CASATI, S. CERUTTI, F. LOMBARDI, M. PAGANI, AND A. MALLIANI. Sympathetic predominance in essential hypertension: a study employing spectral analysis of heart rate variability. *J. Hypertens.* 6: 711–717, 1988.
19. HADDAD, G. G., H. J. JENG, S. H. LEE, AND T. L. LAI. Rhythmic variations in R-R interval during sleep and wakefulness in puppies and dogs. *Am. J. Physiol.* 247 (*Heart Circ. Physiol.* 16): H67–H73, 1984.
20. HYNDMAN, B. W., R. I. KITNEY, AND B. M. SAYERS. Spontaneous rhythms in physiological control systems. *Nature Lond.* 233: 339–341, 1971.
21. KARLSBERG, R. P., P. A. PENKOSKE, P. E. CRYER, P. B. CORR, AND R. ROBERTS. Rapid activation of the sympathetic nervous system following coronary artery occlusion: relationship to infarct size, site, and haemodynamic impact. *Cardiovasc. Res.* 13: 523–531, 1979.
22. KARLSBERG, R. P., P. E. CRYER, AND R. ROBERTS. Serial plasma catecholamine response early in the course of clinical acute myocardial infarction: relationship to infarct extent and mortality. *Am. Heart J.* 102: 24–29, 1981.
23. KATONA, P. G., J. I. H. FELIX. Respiratory sinus arrhythmia: noninvasive measure of parasympathetic cardiac control. *J. Appl. Physiol.* 5: 801–805, 1975.
24. KOEPCHEN, H. P. History of studies and concepts of blood-pressure waves. In: *Mechanisms of Blood-Pressure Waves*, edited by K. Miyakawa, H. P. Koepchen, and C. Polosa. Berlin: Springer-Verlag, 1984, p. 3–23.
25. LOMBARDI, F., C. CASALONE, P. DELLA BELLA, G. MALFATTO, M. PAGANI, AND A. MALLIANI. Global versus regional myocardial ischaemia: differences in cardiovascular and sympathetic responses in cats. *Cardiovasc. Res.* 18: 14–23, 1984.
26. LOMBARDI, F., G. SANDRONE, S. PERNPRUNER, R. SALA, M. GARIMOLDI, S. CERUTTI, G. BASELLI, M. PAGANI, AND A. MALLIANI. Heart rate variability as an index of sympathovagal interaction after acute myocardial infarction. *Am. J. Cardiol.* 60: 1239–1245, 1987.
27. LOWN, B., A. MALLIANI, AND M. PROSDOCIMI (Eds.). *Neural Mechanisms and Cardiovascular Disease*. Padova: Liviana, 1986.
28. MALLIANI, A. Cardiovascular sympathetic afferent fibers. *Rev. Physiol. Biochem. Pharmacol.* 94: 11–74, 1982.
29. MALLIANI, A., P. J. SCHWARTZ, AND A. ZANCHETTI. A sympathetic reflex elicited by experimental coronary occlusion. *Am. J. Physiol.* 217: 703–709, 1969.
30. PAGANI, M., R. FURLAN, S. DELL'ORTO, P. PIZZINELLI, G. BASELLI, S. CERUTTI, F. LOMBARDI, AND A. MALLIANI. Simultaneous analysis of beat by beat systemic arterial pressure and heart rate variabilities in ambulatory patients. *J. Hypertens.* 3(3): S83–S85, 1985.
31. PAGANI, M., F. LOMBARDI, S. GUZZETTI, O. RIMOLDI, R. FURLAN, P. PIZZINELLI, G. SANDRONE, G. MALFATTO, S. DELL'ORTO, E. PICCALUGA, M. TURIEL, G. BASELLI, S. CERUTTI, AND A. MALLIANI. Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circ. Res.* 59: 178–193, 1986.
32. PAGANI, M., V. SOMERS, R. FURLAN, S. DELL'ORTO, J. CONWAY, G. BASELLI, S. CERUTTI, P. SLEIGHT, AND A. MALLIANI. Changes in autonomic regulation induced by physical training in mild hypertension. *Hypertension Dallas* 12: 600–610, 1988.
33. PEÑÀZ, J. Mayer waves: history and methodology. *Automedica Lond.* 2: 135–141, 1978.
34. POLOSA, C. Central nervous system origin of some types of Mayer waves. In: *Mechanisms of Blood-Pressure Waves,* edited by K. Miyakawa, H. P. Koepchen, and C. Polosa. Berlin: Springer-Verlag, 1984, p. 277–292.
35. POMERANZ, B., R. J. B. MACAULAY, M. A. CAUDILL, I. KUTZ, D. ADAM, D. GORDON, K. M. KILBORN, A. C. BARGER, D. C. SHANNON, R. J. COHEN, AND H. BENSON. Assessment of autonomic function in humans by heart rate spectral analysis. *Am. J. Physiol.* 248 (*Heart Circ. Physiol.* 17): H151–H153, 1985.
36. SAUL, J. P., P. ALBRECHT, R. D. BERGER, AND R. J. COHEN. Analysis of long term heart rate variability: methods, 1/F scaling and implications. In: *Computers in Cardiology*. Silver Spring, MD: IEEE Computer Society, 1987, p. 419–422.
37. SAYERS, B. MCA. Analysis of heart rate variability. *Ergonomics* 16: 17–32, 1973.