Am J Physiol Heart Circ Physiol 288: H1777–H1785, 2005.
First published December 16, 2004; doi:10.1152/ajpheart.00594.2004.

Exploring directionality in spontaneous heart period and systolic pressure variability interactions in humans: implications in the evaluation of baroreflex gain

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Submitted 15 June 2004; accepted in final form 6 December 2004

Nollo, Giandomenico, Luca Faes, Alberto Porta, Renzo Antolini, and Flavia Ravelli. Exploring directionality in spontaneous heart period and systolic pressure variability interactions in humans: implications in the evaluation of baroreflex gain. *Am J Physiol Heart Circ Physiol* 288: H1777–H1785, 2005. First published December 16, 2004; doi:10.1152/ajpheart.00594.2004.—Although in physiological conditions RR interval and systolic arterial pressure (SAP) are likely to interact in a closed loop, the traditional cross-spectral analysis cannot distinguish feedback (FB) from feedforward (FF) influences. In this study, a causal approach was applied for calculating the coherence from SAP to RR ($K_{s\text{-}r}$) and from RR to SAP ($K_{r\text{-}s}$) and the gain and phase of the baroreflex transfer function. The method was applied, compared with the noncausal one, to RR and SAP series taken from 15 healthy young subjects in the supine position and after passive head-up tilt. For the low frequency (0.04–0.15 Hz) spectral component, the enhanced FF coupling ($K_{r\text{-}s} = 0.59 \pm 0.21$, significant in 14 subjects) and the blunted FB coupling ($K_{s\text{-}r} = 0.17 \pm 0.17$, significant in 4 subjects) found at rest indicated the prevalence of nonbaroreflex mechanisms. The tilt maneuver recovered FB influences ($K_{s\text{-}r} = 0.47 \pm 0.16$, significant in 14 subjects), which were stronger than FF interactions ($K_{s\text{-}r} = 0.34 \pm 0.19$, significant in 9 subjects). At the respiratory frequency, the RR-SAP regulation was balanced at rest ($K_{s\text{-}r} = 0.30 \pm 0.18$ and $K_{r\text{-}s} = 0.29 \pm 0.20$, significant in 11 and 8 subjects) and shifted toward FB mechanisms after tilt ($K_{s\text{-}r} = 0.35 \pm 0.19$ and $K_{r\text{-}s} = 0.19 \pm 0.11$, significant in 14 and 8 subjects). The causal baroreflex gain estimates were always lower than the corresponding noncausal values and decreased significantly from rest to tilt in both frequency bands. The tilt-induced increase of the phase lag from SAP to RR suggested a shift from vagal to sympathetic modulation. Thus the importance of nonbaroreflex interactions pointed out the necessity of accounting for causality in the cross-spectral analysis of the interactions between cardiovascular variables in healthy humans.

cross-spectral analysis; coherence and transfer function; cardiovascular regulation; feedback and feedforward mechanisms; nonbaroreflex interactions

SINCE THE INTRODUCTION of the notion that cardiovascular oscillations may be indicative of autonomic nervous system status (3), the analysis of the spontaneous fluctuations in heart rate and blood pressure has been extensively used as a probe for cardiovascular control mechanisms in humans. Particularly, the observation that oscillations in the heart period (RR interval) and the systolic arterial pressure (SAP) are correlated around 0.1 Hz and at the frequency of respiration has prompted many researchers to focus on the interrelationship between these two signals. In this context, the cross-spectral analysis of RR interval and SAP variability series constitutes one of the most widespread tools used to investigate on the coupling mechanisms underlying short-term cardiovascular regulation (5, 8, 9, 11, 34, 35, 39, 41, 42). Although limited by the assumption of linear interactions between the two series, it allows the frequency-domain evaluation of the coupling degree, through the coherence function, and of the gain and phase relations, through the magnitude and the argument of the transfer function over a preselected input-output direction.

According to the major role commonly ascribed to the arterial baroreflex in the regulation of the cardiovascular system, so far the utilization of cross-spectral analysis tools has been mainly addressed to the characterization of feedback (FB) interactions occurring in the direction from SAP to RR interval. Indeed, the magnitude and phase of the RR-SAP transfer function evaluated in different frequency bands have been proposed and widely adopted as "spontaneous" estimates of baroreflex sensitivity (1, 28, 30, 34) and of baroreceptor cardiac reflex latency (7, 9, 31, 39). However, it should be remarked that the existence of a pure causal relationship from SAP to RR interval, which is a basic prerequisite for cross-spectral analysis, is implicitly assumed but is not actually tested by the traditional cross-spectral approach. Nevertheless, in the intact circulation, another contribution to cardiovascular variability, operating in the reverse causal direction from RR interval to SAP, has been revealed in healthy humans and defined as a feedforward (FF) mechanism (20, 25, 26, 35). This direct influence of RR interval on SAP should not be intended as a pure regulatory mechanism but rather as a perturbative one, describing the blood pressure changes that follow RR modifications according to the Starling law and the arterial windkessel. Hence, in physiological conditions, RR interval and SAP are likely to reciprocally affect each other as a result of the presence of both regulatory FB and mechanical FF coupling mechanisms. These observations should lead to reconsider the suitability of cross-spectral analysis for the study of the interactions between heart rate and arterial pressure. Indeed, when interactions in the direction opposite to that under investigation cannot be excluded, the traditional cross-spectral analysis approach may produce misleading results as it would intertwine the totally distinct physiological mechanisms operating on the two pathways of the RR-SAP regulatory loop.

To overcome this limitation, we recently proposed the utilization of a simple modification of the traditional parametric cross-spectral analysis, named causal cross-spectral analysis, which elicits the directional dependencies in the calculation of coherence (33) and transfer function (15) between mutually

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interacting variability series. The method has been shown to produce results comparable with noncausal cross-spectral analysis in case of linear unidirectional interactions and to be capable of describing the coupling and transfer function over the selected direction in case of bidirectional interactions between the two analyzed series. In the present study, the causal cross-spectral analysis was used to characterize in the frequency domain the interactions between RR interval and SAP by focusing exclusively on one of the two pathways of their regulatory loop. Particularly, the calculation of the causal coherence functions from SAP to RR interval and from RR interval to SAP quantified the relative contributions of FB and FF arms of the closed loop (CL) to short-term cardiovascular regulation, whereas the comparison between causal and traditional values of the transfer function from SAP to RR interval assessed the impact of nonbaroreflex interactions on the estimates of spontaneous baroreflex gain. The study was carried out in healthy humans in the supine resting position and after passive head-up tilt. This allowed us to assess the role of causality in the evaluation of coherence and transfer function in a normal physiological condition, in which SAP and RR interval are likely to reciprocally interact, and in a condition of enhanced regulation on the baroreflex pathway, in which the FB interactions from SAP to RR interval should be prevalent.

MODELS AND METHODS

Traditional and Causal Approaches to Cross-Spectral Analysis

The cross-spectral analysis of the variability of RR interval (rr) and of SAP (sap) is performed by estimating the auto- and cross-spectral density functions [$P_{rr}(f)$, $P_{sap}(f)$, and $P_{rr,sap}(f)$] and by combining them to obtain the coherence and transfer function estimates. The coherence function

$$\gamma^2_{sap,rr}(f) = \frac{|P_{rr,sap}(f)|^2}{P_{rr}(f)P_{sap}(f)} \quad (1)$$

is a real-valued function that gives the strength of the linear coupling between synchronous oscillations occurring in RR interval and SAP as a function of frequency. It attains a value between zero, indicating absence of coupling at the frequency f, and unity, indicating full coupling at that frequency. The transfer function describes the transfer of power from sap, considered as input, to rr, considered as output of the investigated open-loop linear system, as:

$$H_{sap,rr}(f) = \frac{P_{rr,sap}(f)}{P_{sap}(f)} \quad (2)$$

taking the modulus and the argument of $H_{sap,rr}(f)$ yields, respectively, the gain function $G_{sap,rr}(f)$, linking the sap change to rr variation, and the phase function $\varphi_{sap,rr}(f)$, which is related to the delay between sap and rr oscillations at the frequency f.

The traditional approach to cross-spectral analysis is based on substituting into *Eqs. 1* and *2* the estimates of auto- and cross-spectral density functions derived from either classical Fourier transform-based methods (11) or parametric autoregressive (AR) techniques (5). It is worth noting that the coherence and transfer function obtained with this approach are estimated by considering the effects present on both arms of the RR-SAP regulatory loop (15, 33). As a consequence, the coherence cannot indicate whether an observed degree of coupling is derived from FB influences from sap to rr, from FF influences from rr to sap, or both. Moreover, the transfer function from sap to rr may be corrupted by the possible presence of a reverse FF modulation.

The limitations arising when the traditional cross-spectral analysis is applied to series that interact in a reciprocal manner may be overcome by taking advantage of the concept of causality. According to the definition of causality proposed by Granger (16), given a pair of series such as sap and rr, sap causes rr if sap is useful to predict rr. According to Porta et al. (33), this situation will be taken in the following as an indication of interaction in the direction from sap to rr through the baroreflex FB, whereas the reverse situation (i.e., rr is useful to predict sap) as an indication of interaction in the reverse FF direction through the mechanical coupling. The causal approach is based on performing cross-spectral analysis through the parametric AR technique and then on imposing causality by switching off, before the computation of the coherence and transfer function the pathway of the RR-SAP CL that is not under consideration (33). In detail, the variability of RR interval and SAP is first described by means of the bivariate AR model depicted in Fig. 1, in which the current value of a variability series (rr or sap) is predicted from its own past values (through $A_{rr\text{-}rr}$ or $A_{sap\text{-}sap}$ blocks), from the samples of the other series (through $A_{rr\text{-}sap}$ or $A_{sap\text{-}rr}$ blocks), and from other independent sources (represented by the white noise signals w_{rr} and w_{sap}). After the CL identification of the model (6), the coefficients of the block describing the interactions occurring in the direction opposite to that under analysis are set to zero before the auto- and cross-spectral density functions are computed. In this way, the resulting causal coherence (33) and causal transfer function (15) are estimated by considering only the information exchanged by the two series in the direction under investigation. With regard to the RR-SAP closed loop system, the causal coherences from SAP to RR interval [$\gamma^2_{sap\rightarrow rr}(f)$, measuring the correlation in the direction from sap to rr or the degree of FB coupling] and from RR interval to SAP [$\gamma^2_{rr\rightarrow sap}(f)$, measuring the correlation in the direction from rr to sap or the degree of FF coupling] are defined by forcing to zero the block $A_{sap\text{-}rr}$ and the block $A_{rr\text{-}sap}$ in Fig. 1, respectively. Similarly, the causal transfer function from SAP to RR interval, which estimates the gain [$G_{sap\rightarrow rr}(f)$] and the phase [$\varphi_{sap\rightarrow rr}(f)$] along the FB baroreflex pathway, is defined by setting to zero the block $A_{sap\text{-}rr}$.

It should be remarked that, when the pathway from SAP to RR interval is considered, the coherence $\gamma^2_{sap,rr}(f)$ (under the hypothesis of open-loop interaction) and the causal coherence $\gamma^2_{sap\rightarrow rr}(f)$ (even in CL conditions) both reflect the relative importance of the arterial baroreflex with respect to other nonbaroreflex mechanisms in controlling the sinus node. Indeed, high coherence values indicate that a large part of RR interval variability is due to SAP variability, whereas low coherences are the expression of factors determining the RR variability without affecting SAP and prevailing on the baroreflex.

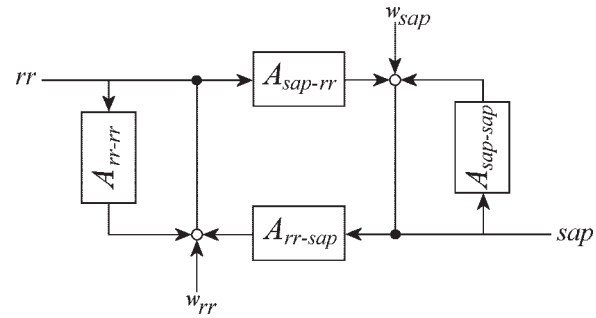

Fig. 1. Bivariate autoregressive model for the description of the interactions between RR interval and systolic arterial pressure (SAP) variability series. The zero-mean series of RR interval (rr) and SAP (sap) are the result of self-influences (modeled by the blocks $A_{rr\text{-}rr}$ and $A_{sap\text{-}sap}$), mutual influences (modeled by the blocks $A_{rr\text{-}sap}$ and $A_{sap\text{-}rr}$), and effects of independent noise sources (represented by the white noise signals w_{rr} and w_{sap}).

Methods

Subjects and experimental protocol. Fifteen healthy young subjects (8 men and 7 women, 25 ± 3 yr old) participated in the study. All subjects were free from any known disease based on anamnesis and physical examination at the time of the study.

The experiments were performed at the Cardiology Division of the Santa Chiara Hospital of Trento, Trento, Italy. Cardiovascular signals were acquired in the morning in comparably comfortable and quiet ambience conditions, with subjects in sinus rhythm and breathing spontaneously. Subjects were positioned in the supine position on a motorized tilt table. After 15 min was allowed for subject stabilization, data were collected during a 10-min quiet, resting baseline period. The table was then tilted to the 60° position, and the acquisition continued for 10 min with subjects in the passive head-up condition.

Data collection and preprocessing. The surface ECG (lead II), finger photopletismographic arterial blood pressure (Finapres, Ohmeda 2300; Englewood, CO), and respiratory activity (by differential pressure transducer) were acquired and digitized with a 1-kHz sampling rate and 12-bit resolution. The beat-to-beat series of the heart period (RR interval) and SAP were then automatically measured on the digitized signals. The series were finally cleaned up from artifacts, windowed to 300 points, and detrended to fulfill stationarity criteria. The variability series were aligned so that the *i*th SAP value occurred within the *i*th RR interval.

Data analysis. For each pair of RR interval and SAP variability series, the traditional and causal cross-spectral analyses were performed by means of the parametric AR approach. First, the two series were reduced to zero mean sequences (rr and sap) to allow for parametric identification (18). The coefficients of the bivariate AR model of Fig. 1 were then estimated from the zero-mean series rr and sap by means of the least-squares approach described in Ref. 6. The model order was searched in the range 6–14 by means of the Akaike's criterion for multivariate processes (2), whereas the whiteness and uncorrelation of the model residuals were checked by means of the Anderson test (18). The canonical form necessary for CL identification was imposed by allowing one beat's delay from rr to sap and an immediate effect from sap to rr (6).

The estimated model coefficients were then used to calculate the spectral and cross-spectral density functions of the sap and rr processes, which in turn were used to compute the traditional and causal estimates of the coherence and transfer functions as described above. Because the two major rhythms commonly studied in cardiovascular variability analysis are those occurring in the low-frequency (LF; 0.04–0.15 Hz) and high-frequency (HF; 0.15–0.4 Hz) bands of the Fourier spectrum (38), the coherence and transfer function estimates were sampled in correspondence with the maximum coupling occurring between RR interval and SAP inside these two frequency ranges.

Assessment of the type of interaction between RR interval and SAP. To establish the nature of the link between RR interval and SAP, we referred to Granger's definition of causality, stating that a series is causal to another if the prediction of the second is improved by incorporating the knowledge of the first (16). In the RR-SAP closed loop model of Fig. 1, sap is causal to rr if at least one of the coefficients of the $A_{rr\text{-}sap}$ block is nonzero and rr is causal to sap if at least one of the coefficients of the $A_{sap\text{-}rr}$ block is nonzero. This means, thanks to the direct relation existing between the model blocks and the causal coherence functions (33), that a causal relationship from one series to the other exists at a given frequency only when the corresponding causal coherence is significantly larger than zero. Thus we classified the interactions between RR interval and SAP in four different types on the basis of the significance of the causal coherences. In each frequency band, open-loop relations were identified when only one of the two causal coherences was significant. Particularly, a FB influence of SAP on RR interval was detected when $\gamma^2_{sap\to rr}$ was significant and $\gamma^2_{rr\to sap}$ was not significant, whereas the opposite situation ($\gamma^2_{rr\to sap}$ significant and $\gamma^2_{sap\to rr}$ not significant) pointed out a FF link from RR interval to SAP. Moreover, a CL relationship was identified when both $\gamma^2_{sap\to rr}$ and $\gamma^2_{rr\to sap}$ were significant, whereas no interactions (NO) were detected when both causal coherences were not significantly exceeding zero.

Because in practical spectral estimation it is very common to observe coherence and causal coherence values higher than zero even in the absence of correlation between the two considered series, a statistical approach for determining the significance of the coherence estimates was utilized (14, 33). With this approach, a threshold for zero coherence was determined specifically for each pair of investigated RR interval and SAP series. First, 100 pairs of surrogate series mimicking the individual properties of the original, but being otherwise completely uncoupled, were generated. The coherence was then estimated for each surrogate pair, and the upper limit of the coherence distribution derived for the surrogate pairs was taken as the threshold function above which the coherence estimates can be considered as significantly larger than zero (14). Examples of calculation of the threshold for zero coherence in different conditions are reported in Fig. 2 (dotted lines).

The surrogate data approach was also exploited to establish the acceptability of the estimates of gain and phase in different conditions in the two frequency bands. Thus traditional and causal transfer function estimates were considered only when the corresponding coherence or causal coherence values were higher than the zero-level threshold.

Statistical analysis. The results relevant to the coupling analysis were interpreted both by sampling the coherence and causal coherence functions at LF and HF and by counting the number of subjects for

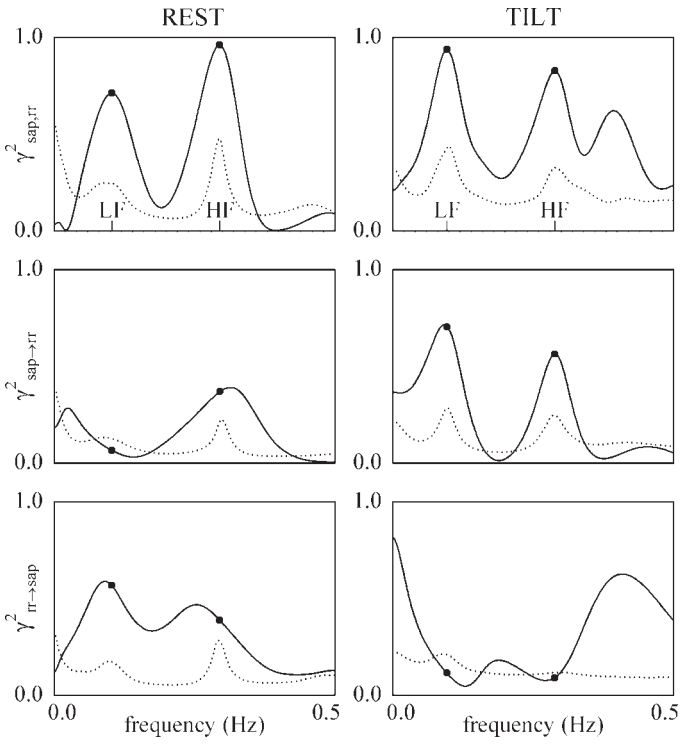

Fig. 2. Representative example of coherence between RR interval and SAP ($\gamma^2_{sap,rr}$; *top*), causal coherence from SAP to RR interval ($\gamma^2_{sap\to rr}$; *middle*), and causal coherence from RR interval to SAP ($\gamma^2_{rr\to sap}$; *bottom*) estimated for a healthy subject in the supine position (*left*) and after head-up tilt (*right*). Solid circles indicate the values of coherence and causal coherences sampled at the frequency of the maximum coupling between RR interval and SAP inside low-frequency (LF) and high-frequency (HF) bands. The dotted lines represent the frequency-dependent thresholds for zero coherence estimated by the surrogate data approach as in Ref. 14.

Table 1. *Analysis of the coupling between RR interval and SAP*

	$\gamma^2_{sap,rr}$	$\gamma^2_{sap\to rr}$	$\gamma^2_{rr\to sap}$	FB	CL	FF	NO
LF							
Rest	0.75±0.13	0.17±0.17‡	0.59±0.21	1	3	11	0
Tilt	0.90±0.06*	0.47±0.16*	0.34±0.19*	6	8	1	0
HF							
Rest	0.76±0.19	0.30±0.18	0.29±0.20	7	4	4	0
Tilt	0.72±0.18	0.35±0.19†	0.19±0.11	7	7	1	0

Values are means ± SD. $\gamma^2_{rr,sap}$ global coherence; $\gamma^2_{sap\to rr}$, causal coherence from systolic arterial pressure (SAP) to RR interval; $\gamma^2_{rr\to sap}$, causal coherence from RR interval to SAP; FB, feedback; CL, closed loop; FF, feedforward; NO, no interactions; LF, low frequency; HF, high frequency. *$P < 0.001$ vs. rest; †$P < 0.05$ and ‡$P < 0.001$, $\gamma^2_{sap\to rr}$ vs. $\gamma^2_{rr\to sap}$ (by paired Student's *t*-test).

which these functions resulted as statistically significant. The transfer functions were analyzed by comparing the distributions over subjects of noncausal and causal estimates sampled at LF and HF for the two body positions.

The statistical significance of the differences in coherence and causal coherences between rest and tilt conditions was assessed by means of the Student's *t*-test for paired data. The same statistical test was used to compare, within a given condition, the two causal coherences or the two methods for estimating the transfer function. Because of the possibly different composition of the relevant distributions, the unpaired Student's *t*-test was chosen to assess differences between rest and tilt for a given transfer function estimate.

The nonparametric Pearson χ^2-test with Yates' correction for 2 × 2 contingency tables was used to assess, given the frequency band and the condition, the statistical significance of differences between the number of subjects showing significant coupling on the baroreflex pathway (FB + CL conditions) and on the nonbaroreflex one (FF + CL conditions). Moreover, the McNemar χ^2-test with continuity correction was applied to verify the hypothesis that prevalence of changes in baroreflex and nonbaroreflex coupling were attributable to the tilt maneuver and not to chance. For all tests, $P < 0.05$ was considered as statistically significant.

RESULTS

Response to Orthostatic Stimulation

The positive response to head-up tilt was documented in the considered healthy subjects by the baroreceptor-induced tachycardia for the maintenance of the average pressure values. On average, the tilt maneuver produced a significant decrease of the mean heart period (rest: 873 ± 91 ms, tilt: 703 ± 62 ms, $P < 0.001$), whereas the SAP values were not substantially modified (rest: 118 ± 20 mmHg, tilt: 114 ± 13 mmHg). Moving from rest to tilt, the variance of the RR interval remained practically unmodified (2,152 ± 1,893 vs. 2,360 ± 1,896 ms^2), whereas that of the SAP series showed a tendency to increase (33 ± 29 vs. 47 ± 25 $mmHg^2$).

Analysis of the Coupling between SAP and RR Interval

Figure 2 shows an example of calculation of the traditional and causal coherence functions in the supine position and during head-up tilt. At rest, the coherence between RR interval and SAP shows two well-resolved peaks with maximum values occurring in the LF and HF bands of the frequency spectrum. In correspondence, the causal coherence from SAP to RR interval is above the threshold for significance only at HF, whereas it is not significant at LF. Differently, the link on the reverse path is strong in both frequency bands, determining a FF condition in the LF band and a CL condition in the HF band. After the tilt maneuver, the global coherence remains very high in the two frequency bands. The causal analysis demonstrates that the strong link in both LF and HF bands is now due to a FB relation between the two series, as the coherence from SAP to RR interval is very high and the coherence from RR interval to SAP is not significant.

The results of coherence and causal coherence analyses extended to the 15 considered subjects are summarized in Table 1 and Fig. 3. Table 1 shows the coherence values expressed as means ± SD and the number of subjects for which the four conditions (i.e., FB, CL, FF, and NO) were detected for the two frequency bands and the two body posi-

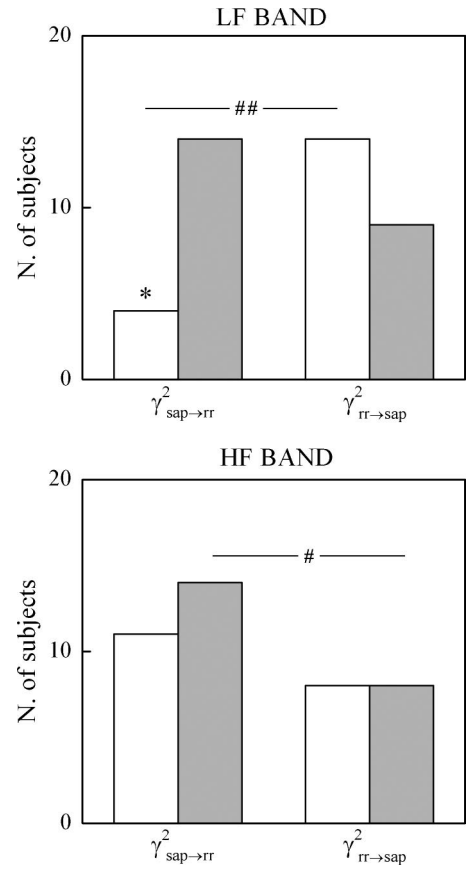

Fig. 3. Assessment of the type of interactions between RR interval and SAP. Bar graphs show the number of subjects (*N*) for which the causal coherence from SAP to RR interval ($\gamma^2_{sap\to rr}$) or from RR interval to SAP ($\gamma^2_{rr\to sap}$) resulted as significant according to the surrogate data approach (14), moving from rest (open bars) to tilt (shaded bars) in the LF band (*top*) and in HF band (*bottom*). *$P < 0.05$ vs. tilt (McNemar χ^2-test); #$P < 0.05$ and ##$P < 0.001$, $\gamma^2_{sap\to rr}$ vs. $\gamma^2_{rr\to sap}$ (Pearson χ^2-test).

tions. In correspondence, Fig. 3 shows the number of subjects showing significant causal coupling from SAP to RR interval (FB + CL conditions) or vice versa (FF + CL conditions). The noncausal coherence calculated by the traditional approach was always high and above the threshold for significance, showing that RR interval and SAP series are significantly correlated in both frequency bands at rest and after tilt. In confirmation of this result, the causal analysis revealed that NO = 0, that is, in all cases at least one of the two causal coherences was larger than the threshold for significance.

In the supine position, the causal coherence from SAP to RR interval evaluated at LF was very low, resulting as significant only in 4 of 15 subjects. Conversely, the causal coherence from RR interval to SAP was statistically higher and exceeded the zero-level threshold in a significantly larger number of subjects (14 of 15 subjects). In a large majority of them (11 of 14 subjects), a pure FF regulation from RR interval to SAP was documented.

The baroreflex regulation in the LF band was substantially recovered by the tilt maneuver, as demonstrated by the significantly increased values of the corresponding causal coherence from SAP to RR interval and by the high number of subjects for which the causal coupling was negligible at rest and became significant after tilt (10 of 11 subjects). In correspondence, the causal coherence on the nonbaroreflex pathway was significantly decreased, even though the reduction of the number of subjects showing coupling was not statistically significant.

In the HF band, the RR-SAP regulation was substantially balanced at rest, as documented by the comparable coherence values and number of subjects with significant coupling on the two pathways. Even though the modifications of coherence values and subjects' proportions were not statistically significant, the tilt maneuver strengthened the baroreflex coupling and blunted the nonbaroreflex one. These variations shifted the RR-SAP regulation toward baroreflex activity. Indeed, after tilt, the causal coherence and the number of coupled subjects were statistically higher on the baroreflex pathway than on the nonbaroreflex one.

Analysis of the Baroreflex Transfer Function

Figure 4 shows the calculation of the transfer function on the baroreflex path (i.e., from SAP to RR interval) performed by traditional and causal approaches for the same series used to estimate the coherence curves of Fig. 2. In the supine position, the causal and global transfer function estimates differ both in terms of gain and phase at LF and only in terms of gain at HF. After tilt, LF and HF values of gain and phase from SAP to RR interval obtained by the causal approach are very close to those derived by the traditional approach.

In Fig. 5, the values of the baroreflex transfer function estimated by the causal approach on subjects exhibiting a non-negligible link from SAP to RR interval (i.e., a FB or CL condition) are compared with the corresponding noncausal estimates. In all subjects, the gain estimates were lower for the causal method than for the traditional noncausal one. The difference between the two estimation procedures resulted as statistically significant in both conditions and frequency bands. The phase values calculated by the causal method in the LF band were significantly less negative than the noncausal estimates both at rest and after tilt. In the HF band, the phases were comparable in the supine position and significantly more negative for the causal approach in the head-up position.

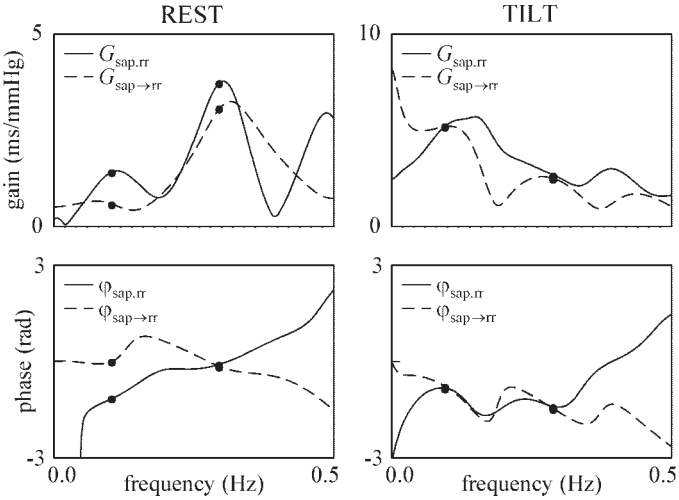

Fig. 4. Representative example of gain (G; *top*) and phase (φ; *bottom*) of the transfer function from SAP to RR interval estimated by the traditional noncausal approach (solid lines) and by the novel causal approach (dashed lines) for a healthy subject in the supine position (*left*) and after head-up tilt (*right*). Solid circles indicate the transfer function values sampled at the frequency of the maximum coupling between RR interval and SAP inside LF and HF bands.

Table 2 describes the response to the orthostatic maneuver of the transfer function from SAP to RR interval estimated by the proposed causal approach when the coupling on the baroreflex pathway was significant. With a shift to the head-up position, the causal estimates of the baroreflex gain decreased significantly in both LF and HF bands. Concerning phase values, at rest the shift from SAP to RR fluctuations resulted more negative in the LF than HF band. Such a negative phase was increased in both bands by the tilt maneuver, with a difference that reached the statistical significance only at HF.

DISCUSSION

The main goal of the present study was to investigate on the role of causality in the interactions between the spontaneous fluctuations of RR interval and SAP in physiological conditions. The concept of causality becomes determinant when the two variables under analysis do not clearly cooperate in an open-loop way along a known direction, as may happen in cardiovascular variability where RR interval and SAP are likely to affect each other as a result of the FB and FF contributions to cardiovascular regulation (22). The computation of the causal coherence (33), along with the accurate determination of the significance of the link between RR interval and SAP in a given frequency band (14), allowed us to distinguish four different patterns of interaction between the two investigated variability series: FB, when a significant coupling was detected only on the baroreflex pathway from SAP to RR interval; FF, when only the nonbaroreflex pathway from RR to SAP was active; CL, when a significant coupling was found on both causal directions; and NO, when the coupling was negligible on both pathways. In addition, with the causal transfer function (15), it was possible to discard the biasing effects of possible nonbaroreflex interactions from the

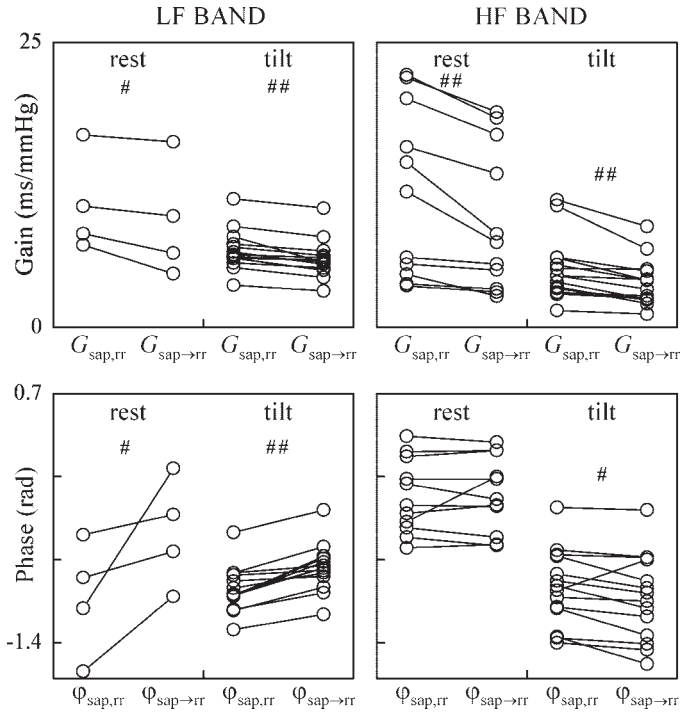

Fig. 5. Comparison between causal and noncausal estimates of the baroreflex transfer function. Plots report the gain (*top*) and phase (*bottom*) values estimated at LF (*left*) and HF (*right*) by the noncausal approach ($G_{sap,rr}$ and $\varphi_{sap,rr}$) and by the causal method ($G_{sap\to rr}$ and $\varphi_{sap\to rr}$) in rest and tilt conditions. $^{\#}P < 0.05$ and $^{\#\#}P < 0.001$, causal vs. noncausal estimates (paired Student's *t*-test).

estimation of the baroreflex response in spontaneous conditions.

When the two commonly observed oscillations of RR interval and SAP (i.e., those occurring at the frequency of the Mayer waves and that at the respiratory frequency) are considered, the use of causality may shed further light in the understanding of the physiological mechanisms involved in cardiovascular regulation. Particularly, our results at LF induce some considerations about the generation of the slow oscillation in blood pressure and heart rate, which is one of the most contentious aspects of cardiovascular variability (23, 36). The large number of FF interactions found in the LF band at rest indicates that, in this condition, the high coherence always observed between RR interval and SAP is mostly due to a coupling from RR interval to SAP. This result is in agreement with that found by Porta et al. (33) in an older healthy population, thus suggesting that directionality in SAP and RR interactions at LF is not modified by aging. Hence, at the frequency of the Mayer waves the influences of heart period on SAP seem to be prevalent over the well-studied baroreflex regulation of heart rate. This finding confirms for the LF oscillations the mounting impression that nonbaroreflex or nonautonomic mechanisms play a major role in the regulation of RR interval and SAP spontaneous fluctuations (13, 22, 24, 26). Even though the hypothesis that nonbaroreflex interactions might represent the expression of a neurally mediated cardiovascular regulation has been recently raised (20), the most common interpretation about this branch of the RR-SAP CL assumes that heart rate mechanically feeds forward to produce parallel changes in arterial pressure through cardiac output (25). According to this view, the reciprocal CL influence between heart rate and arterial pressure could be interpreted as a continuous interplay between regulation, accomplished by the FB neural pathway, and perturbation, due to the FF mechanical pathway. Hence, despite the actual meaning of the term, the presence of a FF coupling from RR interval to SAP does not necessarily indicate the involvement of a proactive function in the regulation. More probably, FF coupling mechanisms just account for blood pressure changes driven by modifications in the RR interval that in turn may be determined in a reactive way, either through the FB mechanism or through nonbaroreflex influences of central and/or peripheral origin.

Besides the strength of nonbaroreflex interactions, our results in the LF band evidenced for the supine subjects a very low extent of coupling on the causal path from SAP to RR interval. This finding suggests that mechanisms other than the arterial baroreflex, possibly of central origin (4, 10), are probably involved in the genesis of LF oscillations of heart rate. Moreover, the lack of baroreflex regulation revealed for supine subjects at LF should lead to the reconsideration of the possibility of estimating baroreflex gain from the spontaneous variability of SAP and RR interval measured in the basal resting condition (21). This result underlines the necessity of investigating the baroreflex-mediated changes in the heart rate under different conditions characterized by a more significant baroreflex involvement. One possible explanation for the lack of baroreflex engagement in the LF band may lie in the blunted activity of the baroreceptor regulatory function in the supine condition rather than in its malfunctioning. With subjects in the supine position and after time allowed for stabilization, the low baroreflex involvement may indeed result from nonbaroreflex factors limiting the FB regulation and from central inhibitory influences. Nevertheless, the tilt maneuver was able to produce an activation of the control on the baroreflex path, as documented by the significant increase of the causal coherence from SAP to RR interval and of the number of subjects exhibiting significant coupling.

At the respiratory frequency, causal coherence analysis suggests that both FB and FF pathways contribute to the high coherence in the HF band. This result is in agreement with that found by Porta et al. (33) in an older population, again confirming that aging does not modify the nature of the interactions between heart rate and blood pressure even at HF. It is consistent with the hypothesis that two pathways driven by respiration may be simultaneously active in supine humans: the respiratory-induced changes in heart rate that in turn affect

Table 2. *Estimation of the causal transfer function from SAP to RR interval*

	n	$G_{sap\to rr}$, ms/mmHg	$\varphi_{sap\to rr}$, rad
LF			
Rest	4	9.3±5.1	−0.47±0.46
Tilt	14	6.0±1.7*	−0.77±0.20
HF			
Rest	11	9.4±6.3	−0.13±0.35
Tilt	14	3.9±2.1*	−1.02±0.36†

Values are means ± SD; *n,* no. of subjects in which the causal coupling from SAP to RR was significant according to a surrogate data test (14). $G_{sap\to rr}$ and $\varphi_{sap\to rr}$, gain and phase of the transfer function from SAP to RR evaluated by the causal approach. $^{*}P < 0.05$ and $^{\dagger}P < 0.005$ vs. rest (by Student's *t*-test).

arterial pressure and the mechanical action of respiration on arterial pressure that is propagated to the sinus node through the baroreflex (4, 35). Thus respiratory sinus arrhythmia could be due either to neural modulations projecting the respiratory activity on the sinus node or even to baroreflex-mediated effects of respiration on arterial pressure (17). In the upright position, the increase of coupling on the path from SAP to RR interval and the concomitant decrease on the opposite path suggest that the influences of respiration on arterial pressure triggering the feedback regulation of heart rate may prevail over the modulating effects of respiratory sinus arrhythmia on arterial pressure (12, 39). This larger contribution of the baroreflex mechanism after tilt could be favored by the increased mechanical influences of respiration on SAP in the upright position (4, 35).

The finding that the baroreflex activity affects heart rate regulation to a lesser extent in the supine than standing position, documented by the lower coupling from SAP to RR interval found at rest in both LF and HF bands, may be compared with the reduction of the baroreflex involvement during night previously observed by Di Rienzo et al. (13). This comparison draws a relationship between the causal coherence from SAP to RR interval and the baroreflex effectiveness index (BEI) proposed in Ref. 13. Indeed, the BEI may be considered an index measuring the degree of correlation in the causal direction from SAP to RR interval, as the percentage of baroreflex sequences should increase in the presence of increased correlation along the FB pathway. Therefore, the increase of BEI during the day observed in Ref. 13 may have the same meaning of the increase of correlation in the causal direction from SAP to RR interval during tilt. Nevertheless, it should be pointed out that the causal coherence takes RR interval linear dependencies on both immediate and past SAP samples into account (33) and thus should be capable to consider both fast and slow baroreflex mechanisms inducing changes in the heart period causally correlated to pressure variations. On the contrary, the capability of BEI might be limited by the definition of baroreflex sequences involving only those SAP-RR interval interactions without any memory over several past SAP values (32).

The estimates of the baroreflex gain calculated by the proposed causal method were always significantly lower than those obtained through the traditional cross-spectral approach. This finding is in accordance with that of previous studies (19, 27) in which noncausal estimates were shown to be larger than causal gain indexes derived from parametric models. Therefore, our data confirm the importance of taking the role of causality into account when the baroreflex gain is estimated from spontaneous variabilities. Indeed, the gain function obtained by the traditional cross-spectral method is likely to overestimate the true transfer function modulus as it accounts for FF interactions in addition to the investigated FB ones. On this matter, recent studies developing specific open-loop parametric models (27, 32) showed that gain estimates derived from the traditional bivariate model may be biased in the presence of mechanisms other than the baroreflex, also observing the better adherence of causal gain estimates to clinical baroreflex sensitivity values (27). In addition to the circulatory mechanics, also regulatory mechanisms capable of affecting RR interval independently of the baroreflex circuit [e.g., neural influences projecting the activity of central oscillators, neural reflexes involving low-pressure receptors (40), and cardiogenic reflexes initiated by abnormal situations (17, 32)] should indeed be responsible of the difference between baroreflex gain indexes obtained through a causal modeling approach or through the traditional methods (32).

Despite the different absolute values, the response to tilt observed in the causal baroreflex gain when subjects moved from the lying to standing position is in agreement with that observed in previous studies (9, 37). Indeed, a significant reduction in the gain estimates was documented when subjects moved from the supine to upright position. This result holds for both the considered frequency bands, suggesting that the behavior of the baroreflex is unique, that is, the baroreflex gains in LF and HF bands may have different values, but they undergo consensual changes as a result of the same stimulus. The reduction of gain observed with the tilt maneuver occurred contemporaneously with the coupling increase documented by the higher causal coherence in the standing position. This discrepancy in the behavior of gain and coherence suggests that the two measures convey different information about the baroreflex control of heart rate. While the coherence reflects the relative amount of RR interval variability that can be explained by SAP variability, the gain quantifies, when the baroreflex drive is effective, the magnitude of the reflex RR interval response with respect to the amplitude of the input SAP change. In reaction to the orthostatic stimulus, the coherence increase should reflect the major involvement of the baroreflex in heart rate regulation, which is expected to be due to the modified condition that stimulates the cardiovascular control tending to alter the average pressure level. The contemporaneous reduction in the gain may be explained by considering that the same efficiency in the dynamic adjustment of heart rate, documented by the substantially unmodified RR variance after tilt, is achieved starting from an augmented pressure input (i.e., the SAP variance is significantly increased in the standing position).

Regarding the phase function, it should be remarked that values obtained through the traditional noncausal approach should merely reflect the coexistence of FB and FF effects rather than measuring the delay of FB interactions and thus should not be used to state which signal precedes the other (39) or to quantify the delay of the interaction (9, 31). On the contrary, the causal method, separating FB from FF effects, estimates the phase lag exclusively along the pathway from SAP to RR interval. Hence, the differences between traditional and causal phase estimates should be determined mainly by the relative weight of the FF contribution to cardiovascular regulation. Particularly, at LF in the supine position, the predominance of nonbaroreflex interactions led the causal approach to estimate less negative phase lags than those derived by the noncausal method, which in turn are similar to the phase shifts reported in the literature (5, 9, 12, 39, 41). The less negative phase lags observed in the LF band for the causal method suggest that at LF the SAP variations are more rapidly transferred to the RR interval, probably reflecting the activity of a fast vagal modulation. The average phase shift of about 0.47 rad found in the LF band at rest, which corresponds to a delay of 0.75 s from SAP to RR oscillations at 0.1 Hz, is indeed compatible with the theoretical time of baroreflex latency (29). The increase of the phase shift after tilt, although not statistically significant, could account for the activation of the slow

sympathetic regulation simultaneously with the deactivation of the fast vagal response (22). At HF, the less negative phases from SAP to RR interval with respect to the LF band observed in the supine position support the hypothesis that an intervention of the baroreflex mechanism at respiratory frequencies is possible thanks to the rapid vagal response to baroreceptor stimuli (12). The more negative phases after tilt indicate a slowing of the transmission of SAP changes to RR interval through the baroreflex pathway. This result may be interpreted by considering that shortening the RR interval below the time of baroreflex latency, as can happen after tilt, may prevent SAP changes to be sensed by the sinus node until the subsequent heartbeat, thus resulting in a delayed baroreflex regulation (7). At any rate, more complex trivariate models including respiration (4, 32) should be considered for a complete understanding of the complex cardiovascular interactions occurring in the HF band.

In summary, our study points out the need of considering causality in the analysis of the coupling between the spontaneous fluctuations of heart period and arterial pressure in humans in physiological conditions. The utilization of a causal approach able to distinguish the FB from FF pathway in the estimation of the coherence function evidenced the prevailing role of nonbaroreflex mechanisms, mostly at the frequency of the Mayer waves, in the genesis of the interactions between RR interval and SAP in supine subjects. Furthermore, the results of causal transfer function analysis confirmed that the traditional, cross-spectrum-based evaluation of the gain from SAP to RR interval tends to overestimate the actual spontaneous baroreflex sensitivity and is compatible with a shift from vagal to sympathetic activity of the cardiovascular regulation after passive head-up tilt.

GRANTS

This study was supported by the Instituto Trentino di Cultura of Trento, Italy.

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