Information domain analysis of the spontaneous baroreflex during pharmacological challenges

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1. Introduction

The characterization of the relation between spontaneous heart period (HP) and systolic arterial pressure (SAP) variabilities provided important information about one of the short-term neural reflex essentially contributing to cardiovascular homeostasis, i.e. baroreflex (Di Rienzo et al., 1997). The importance of this technique lies in the possibility to probe baroreflex noninvasively and in more physiological conditions compared to the application of the traditional invasive method based on the administration of vasoactive drugs (Smyth et al., 1969). Usually, noninvasive characterization of baroreflex based on spontaneous HP and SAP variabilities involves the computation of sensitivity, latency and coupling strength of the HP–SAP variability relation in time (Bertinieri et al., 1988; Panerai et al., 1997; Porta et al., 2000a; Westerhof et al., 2006; Silvani et al., 2008; Cividjian et al., 2011) and/or frequency domains (Robbe et al., 1987; Pagani et al., 1988; Cooke et al., 1999; Nollo et al., 2005). This characterization has been used to study cardiovascular control in humans. For example, in healthy subjects the sympathetic activation induced by head-up tilt test causes the decrease of the gain of the HP–SAP variability relation, the increase of the coupling strength in the low frequency band (about 0.1 Hz) and a negative phase shift at the respiratory rate suggesting that HP changes lag behind SAP variations (Cooke et al., 1999; Nollo et al., 2005; Westerhof et al., 2006; Porta et al., 2011). In clinical setting, baroreflex

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sensitivity assesses from spontaneous HP and SAP variabilities provided relevant prognostic information in heart failure (La Rovere et al., 2011).

More recently the HP–SAP variability interactions were described in the information domain (Porta et al., 2000b), thus complementing the analysis in time and frequency domains. This approach is grounded on the measurement of the information carried by HP given SAP changes according to the estimation of cross-conditional entropy (Porta et al., 1999): the larger the information carried by HP given SAP variations, the greater the unpredictability of HP given SAP modifications, the smaller the strength of the causal coupling from SAP series to HP series (Porta et al., 2011). Although the analysis of HP–SAP variability interactions in the information domain provided evidence that the strength of the causal coupling from SAP series to HP series was increased with tilt table inclination during a graded head-up tilt protocol, thus suggesting a progressive involvement of the baroreflex in regulating HP–SAP variability interactions as a function of the magnitude of the gravitation stimulus (Nollo et al., 2002; Porta et al., 2011), no systematic assessment of the information carried by HP given SAP changes during selective vagal and/or sympathetic blockade was performed. This absence prevents the assessment of the role played by the autonomic nervous system in governing the information domain interactions from SAP variability to HP variability. We hypothesize that modifications of the efficiency of the baroreflex induced by the autonomic nervous system result in variations of the information carried by HP given SAP variations.

In addition, the difficulty in predicting HP given SAP variations depends on the forecasting time, defined as the number of steps into the future of HP for which the forecast is made: the longer the forecasting time, the more unreliable the prediction of HP given SAP changes, the larger the information carried by HP given SAP modifications. In univariate analysis it was proven that the rate of the increase of unpredictability with the forecasting time depends on the dynamical properties of the process (e.g. it depends on the largest Lyapunov exponent), thus becoming a parameter that might be important to typify the dynamics of the series (Sugihara and May, 1990; Wales, 1991; Tsonis and Elsner, 1992).

In this specific application involving the assessment of the HP–SAP dynamical interactions the rate of increase of unpredictability with forecasting time might provide a new and helpful parameter to characterize HP–SAP variability relation (Porta et al., 2010).

The aim of the study is to characterize the spontaneous baroreflex as a dynamical relation from SAP series to HP series in the information domain according to the calculation of cross-conditional entropy of HP given SAP changes. The characterization was performed during an experimental protocol capable of modulating baroreflex sensitivity via the administration of drugs blocking sympathetic and/or parasympathetic branches of the autonomic nervous system (Parlow et al., 1995). The information carried by HP given SAP variations was typified according to two parameters: i) the information carried by HP given SAP changes within the same heart cycle (i.e. 0-step-ahead information); and ii) the rate of increase of the information carried by HP given SAP variations as a function of the temporal distance between the conditioning pattern of SAP and future value of HP (i.e. the rate of the increase of the k-step-ahead information with k). While the 0-step-ahead information describes the immediate effect of SAP changes on HP, the rate of the increase of the k-step-ahead information with k elucidates short-term effects of SAP modifications on HP. Information domain indexes were compared with parameters traditionally exploited to typify spontaneous baroreflex (i.e. baroreflex sensitivity and squared HP–SAP coherence).

2. Materials and methods

2.1. Pharmacological challenges

This protocol was originally designed to study the effects of pharmacological blockades of the parasympathetic and sympathetic branches of the autonomic nervous system on the cardiac baroreflex sensitivity (Parlow et al., 1995; Toader et al., 2008). Briefly, we studied 9 healthy male physicians aged from 25 to 46 years familiar with the study setting. None of them had any abnormal finding in history, physical examination or electrocardiography or was receiving any medication. All had normal resting brachial arterial pressure measured by sphygmomanometer. They were instructed to avoid tobacco, alcohol and caffeine for 12 h and strenuous exercise for 24 h before each experiment. Electrocardiogram (ECG) and noninvasive finger blood pressure (Finapress 2300, Ohmeda, Englewood, Colorado, USA) were recorded during the experiments. The hand of the subject was kept at the level of the heart. Signals were sampled at 500 Hz. Experimental sessions were performed in 3 days at approximately 2 week intervals. One volunteer took part only in the first day experiments. Subjects remained at rest in supine position in a quiet darkened room during all the recordings. Each experiment started in the morning between 08:00 and 09:00 AM and consisted of 15–20 min of baseline (B) recording followed by 15–20 min of recording after drug administration. Recordings were obtained: i) on day 1 after parasympathetic blockade with 40 μg·kg⁻¹ i.v. atropine sulfate (atropine, AT) to block muscarinic receptors; ii) on day 2 after β-adrenergic blockade with 200 μg·kg⁻¹ i.v. propranolol (PR) to block β₁ cardiac and β₂ vascular peripheral adrenergic receptors; iii) on day 1 PR was administered at the end of the AT session (the dose of AT was reinforced by 10 μg·kg⁻¹) to combine the effect of AT and PR (AT + PR) and obtain a cardiac parasympathetic and sympathetic blockade; iv) on day 3 recordings were obtained 120 min after 6 mg·kg⁻¹ per os clonidine hydrochloride (clonidine, CL) to centrally block the sympathetic outflow to heart and vasculature. All the subjects gave their written informed consent. The protocol adhered to the principles of the Declaration of Helsinki. The human research and ethical review board of the Hospices Civils de Lyon approved the protocol.

2.2. Extraction of the beat-to-beat variability series

After detecting the QRS complex on the ECG and locating the R-apex using parabolic interpolation, the temporal distance between two consecutive R parabolic apexes was computed and utilized as an approximation of HP. The maximum of arterial pressure inside the i-th HP (i.e. HP(i)) was defined as the i-th SAP (i.e. SAP(i)), where i is the progressive cardiac beat counter. The occurrences of QRS and SAP peaks were carefully checked to avoid erroneous detections or missed beats. If isolated ectopic beats affected HP and SAP values, these measures were linearly interpolated using the closest values unaffected by ectopic beats. HP and SAP were extracted on a beat-to-beat basis. The series were linearly detrended. Sequences of 256 consecutive measures were randomly selected inside each experimental condition. If evident nonstationarities, such as very slow drifting of the mean or sudden changes of the variance, were present despite the linear detrending, the random selection was carried out again.

2.3. Normalized uncoupling index from SAP series to HP series at a given forecasting time

Defined the current HP value as HP(i) and the SAP pattern formed by the current SAP value, SAP(i), and by the L-1 past SAP values as SAPL(i) = (SAP(i), SAP(i−1),..., SAP(i−L+1)), HP = {HP(1), i = 1,..., N}, SAP = {SAP(i), i = 1,..., N} and SAPL = {SAP(i), i = 1,..., N} denotes the series of HP values, SAP values and SAP patterns of length L, where N is the size of the series. The cross-conditional entropy of HP given SAPL at the forecasting time k (CCEHP→SAP(L,k)) measures the remaining average uncertainty about HP(i+k) when SAPL(i) is given (Porta et al., 1999, 2010), with k measures how far prediction of HP is made into the future. It is bounded between 0 and the average uncertainty associated to HP when SAP is not given, quantified by the
Shannon entropy of HP ($S_{EHP}$). If HP is completely predictable based on SAP, the residual uncertainty is null (i.e. $CCE_{HPSAP}(L) = 0$). Conversely, if HP is totally unpredictable (i.e. SAP is not helpful to predict HP), the residual uncertainty is maximal (i.e. $CCE_{HPSAP}(L) = S_{EHP}$). $CCE_{HPSAP}(L)$ depends on the forecasting time $k$. Assessed $L$, $CCE_{HPSAP}(L)$ increases with $k$ because prediction of HP becomes more and more unreliable with $k$ unless HP is completely predictable or unpredictable based on SAP changes. Assessed $L$, the rate of increase of $CCE_{HPSAP}(L)$ with $k$ depends on the dynamical properties of the HP–SAP variability relation.

We followed the approach set in Porta et al. (1999) to estimate $CCE_{HPSAP}(L)$. Briefly, the HP and SAP series were coarse-grained according to a uniform quantization procedure. Under this quantization technique the full range of SAP and HP dynamics (i.e. the difference between the maximum and minimum value) was spread over $\xi = 6$ quantization bins. Uniform quantization imposed a partition of the L-dimensional space, where the patterns SAPL were embedded, into $\xi^L$ nonoverlapping hyper-cubes whose reunion covered the entire space. Partition allowed the approximation of the condition probabilities of HP$(i + k)$ given SAPL$(i)$ with the conditional sample frequencies and, thus, the calculation of $CCE_{HPSAP}(L)$. Unfortunately, assessed $k$, $CCE_{HPSAP}(L)$ always decreased to 0 as a function of $L$ independently of the ability of SAPL to reduce the uncertainty of HP. This decrease was solely the effect of the finite amount of samples utilized to assess $CCE_{HPSAP}(L)$ in association to the need of assessing conditional sample frequencies at progressively increasing embedding dimension $L$. In order to counteract this bias, we exploited the estimator, referred to as correct $CCE_{HPSAP}(L)$ ($CCE_{HPSAP}(L)$) defined in (Porta et al., 1999) preventing the decrease to 0 of $CCE_{HPSAP}(L)$ by substituting the null information associated to HP$(i + k)$ given SAPL$(i)$ when it is likely to be the result of smallness of the series with the maximum amount of information carried by HP (i.e. $S_{EHP}$). As a result $CCE_{HPSAP}(L)$ decreased towards 0 with $L$ only in the case that HP was perfectly predictable given SAP changes, it remained constant when SAP variations was not helpful to predict HP, and it exhibited a minimum when SAP variations was only partially helpful to predict HP. The minimum of $CCE_{HPSAP}(L)$ over $L$ divided by $S_{EHP}$ was taken as the best estimate of the normalized residual amount of uncertainty associated to a value of HP $k$ steps into the future of SAP: the higher this minimum, the bigger the k-step-ahead information carried by HP given SAP changes, the larger the k-step-ahead unpredictability of HP given SAP variations, the smaller the strength of the causal k-step-ahead coupling from SAP variability to HP variability. The minimum of $CCE_{HPSAP}(L)$ over $L$ normalized by $S_{EHP}$ will be referred to as normalized k-step-ahead uncoupling index from SAP series to HP series ($NUCI_{HP,SAP}(k)$) in the following. Mostly $NUCI_{HP,SAP}$ has been assessed with $k = 0$ (Porta et al., 1999; Faes et al., 2011; Porta et al., 2011), here indicated as $NUCI_{HP,SAP}$, thus estimating the average amount of information carried by HP given SAP changes within the same heart cycle (i.e. 0-step-ahead information). In this study $NUCI_{HP,SAP}$ (i.e. the k-step-ahead information) was monitored as a function of the forecasting time $k$. The rate of average increase of uncertainty (i.e. loss of predictability) with $k$ was estimated as the slope of the regression line of $NUCI_{HP,SAP}$ on $k$ (Porta et al., 2010).

2.4. Type-1 and type-2 surrogates

We tested two null hypotheses: i) the inability of SAP to reduce the uncertainty associated to HP (i.e. the null hypothesis of HP–SAP uncoupling); and ii) the inability of SAP to reduce the uncertainty of SAP above and beyond linear HP–SAP variability interactions (i.e. the null hypothesis of linear HP–SAP variability relation). The null hypothesis of HP–SAP uncoupling was tested by creating for each pair of original HP and SAP series one pair of iso-distributed iso-spectral surrogate series. The surrogate series preserved distributions and power spectra of the original series, while phases were rotated by uniformly distributed random numbers ranging from 0 to $2\pi$ (Theiler et al., 1992). An iteratively-refined amplitude-adjusted Fourier transform (IAAFT) procedure was exploited (Schreiber and Schmitz, 1996). IAAFT surrogates preserved exactly distribution of the original series, while the power spectrum was the best approximation of the original power spectrum according to the number of iterates (here 100). Since two independent random sequences were utilized to randomize phases of HP and SAP series, the resulting HP and SAP surrogates were uncoupled (i.e. the two surrogate series oscillated asynchronously) (Palus, 1997).

This type of surrogates will be referred to as type-1 surrogates in the following. The null hypothesis that HP–SAP variability interactions could be exclusively explained by a linear HP–SAP variability relation was tested by creating again one pair of iso-distributed iso-spectral phase-randomized surrogates for each pair of original HP and SAP series according to the IAAFT technique. At difference with type-1 surrogate, the phase of HP surrogate was substituted with the phase of the SAP surrogate augmented by the phase difference between HP and SAP original series, thus preserving the cross-spectrum phase of the original series in the surrogate ones (Pritchard and Theiler, 1994). Since the power spectra of both HP and SAP series were preserved as well, their cross-spectrum was entirely maintained and, thus, also their cross-correlation function. This type of surrogates will be referred to as type-2 surrogates in the following. The null hypotheses were rejected when $NUCI_{HP,SAP}$ assessed over the original data was found significantly smaller than that computed over surrogates. The rejection of the null hypothesis of HP–SAP uncorrelation means that the reduction of uncertainty of HP due to the introduction of SAP is significant (i.e. there is a significant causal coupling from SAP variability to HP variability). The rejection of the null hypothesis that HP–SAP variability interactions can be fully described by a linear relation means that the reduction of the uncertainty of HP due the introduction of SAP is not significant and cannot be explained solely by the linear properties of the HP–SAP variability relation and a significant nonlinear coupling from SAP series to HP series can be hypothesized.

2.5. Traditional tools for the assessment of the spontaneous baroreflex

Spectral and cross-spectral analyses were carried out to provide a traditional assessment of the baroreflex from spontaneous beat-to-beat HP and SAP variabilities (De Boer et al., 1985; Pagani et al., 1988; Baselli et al., 1997).

Spectral analysis allowed the estimation of baroreflex sensitivity as the square root of the ratio of the HP power to the SAP power in the low frequency (LF) and high frequency (HF) bands (Pagani et al., 1988). These two indexes will be labeled as $\alpha$(LF) and $\alpha$(HF) in the following. Power spectral density was estimated after fitting the HP and SAP series with an autoregressive (AR) model (Pagani et al., 1988). The Levinson–Durbin recursive algorithm was utilized to estimate the coefficients of the AR model and the variance of the white noise. The number of coefficients was chosen according to the Akaike’s figure of merit. Power spectral density was computed from the AR coefficients and from the variance of the white noise. The power spectral density was factorized into spectral components accounting for the total variance of the series (Pagani et al., 1988). A spectral component was labeled as LF if its central frequency was between 0.03 and 0.15 Hz, while it was classified as HF if its central frequency was between 0.15 and 0.4 Hz. The LF and HF powers were defined as the sum of the powers of all LF and HF spectral components respectively and utilized to calculate $\alpha$(LF) and $\alpha$(HF).

Cross-spectral analysis allowed the quantification of the degree of linear association between HP and SAP series as a function of the frequency via the squared coherence function ($K_{HP,SAP}^2$). $K_{HP,SAP}^2$ was computed as the ratio of the squared HP–SAP cross-spectrum modulus to the product of the power spectra of HP and SAP series (De Boer et al., 1985). $K_{HP,SAP}^2$ ranged from 0 to 1 indicating a perfect uncorrelation and a full association respectively. $K_{HP,SAP}^2$ was estimated via a parametric approach based on bivariate AR model (Baselli et al., 1997). The model order was fixed to 10 and the coefficients of the
bivariate AR model were identified via least squares approach. $K^2_{\text{HP-SAP}}$ was sampled in correspondence of the weighted average of the central frequencies of the LF and HF components found in the SAP series, where the weights were the powers of the components.

### 2.6. Statistical analysis

Kruskal–Wallis one way analysis of variance on ranks was applied over the entire group of subjects to check whether $NUCI^k_{\text{HP-SAP}}$ changed after pharmacological challenge (Dunn’s test for multiple comparisons of AT, AT + PR, PR and CL versus B). Wilcoxon signed rank test was applied over the entire group of subjects to check differences between $NUCI^k_{\text{HP-SAP}}$ derived from original data and surrogate pairs. Linear regression analysis of $NUCI^k_{\text{HP-SAP}}$ on $k$ was carried out in all the experimental conditions. The null hypothesis of slope equal to 0 (i.e. no linear relationship) was tested. If a significant linear relation was found at B, the slope of the regression line was compared with that maybe found at AT, AT + PR, PR and CL and the null hypothesis of equal slope was tested. When a significant linear relation was found over both the original series and surrogate pairs in a given an experimental condition, the null hypothesis of equal slope was checked as well. A $p < 0.05$ was always considered as significant.

### 3. Results

Fig. 1a shows $NUCI^0_{\text{HP-SAP}}$ assessed from the original HP and SAP series as a function of the experimental condition. $NUCI^0_{\text{HP-SAP}}$ is depicted as mean plus standard deviation over the entire group of subjects. No significant difference was found after AT, AT + PR and PR compared to B (Fig. 1a). Conversely, $NUCI^k_{\text{HP-SAP}}$ decreased significantly after CL (Fig. 1a). Comparison between $NUCI^k_{\text{HP-SAP}}$ assessed from the original series (black bars) and surrogate pairs (white bars) is shown in Fig. 1b and c. Results from the original series are contrasted with those derived from iso-distributed iso-spectral uncoupled surrogate pairs in Fig. 1b and from surrogate pairs preserving cross-correlation in Fig. 1c. Also $NUCI^k_{\text{HP-SAP}}$ assessed from surrogate pairs is depicted as mean plus standard deviation over the entire group of subjects. The null hypothesis of uncoupling between HP and SAP series within the same cardiac beat was rejected at B and after PR and CL (Fig. 1b), thus suggesting that the two series were coupled along the causal direction from SAP series to HP series in the above mentioned experimental conditions. The null hypothesis of uncoupling could not be rejected after AT and AT + PR (Fig. 1b). The null hypothesis of linear coupling between HP and SAP series within the same cardiac beat was rejected after CL (Fig. 1c), thus indicating that the HP–SAP correlation, observed at B, after PR and CL (Fig. 1b) could be exclusively explained in terms of a linear relation between HP and SAP series at B and after PR (Fig. 1c), while nonlinear mechanisms significantly contributing to the HP–SAP variability link after CL (Fig. 1c).

Fig. 2 shows the results of linear regression analysis of $NUCI^k_{\text{HP-SAP}}$ on the forecasting time $k$ performed over the original series. A significant linear relationship of $NUCI^k_{\text{HP-SAP}}$ on $k$ was found both at B (Fig. 2a) and after CL (Fig. 2e): indeed, the slope of the linear regression was significantly different from 0 in these two experimental conditions. Correlation coefficient was 0.19 at B and 0.36 after CL. The slopes were positive, thus indicating that $NUCI^k_{\text{HP-SAP}}$ increased with $k$ (i.e. HP became more and more unpredictable given SAP changes with $k$). No significant linear regression of $NUCI^k_{\text{HP-SAP}}$ on $k$ was detected after AT, AT + PR and PR (Fig. 2b, c, and d). The slope of the regression line after CL (Fig. 2e) was significantly steeper than that at B (Fig. 2a).

Linear regression analysis of $NUCI^k_{\text{HP-SAP}}$ on $k$ was carried out over surrogate series as well (Fig. 3): in Fig. 3a, c, e, g, and i $NUCI^k_{\text{HP-SAP}}$ was assessed over uncoupled surrogate pairs, while in Fig. 3b, d, f, h, and j $NUCI^k_{\text{HP-SAP}}$ was computed from surrogate pairs preserving cross-correlation. When $NUCI^k_{\text{HP-SAP}}$ was assessed from uncoupled surrogate pairs, no significant linear relation was found in any considered experimental condition. Conversely, when $NUCI^k_{\text{HP-SAP}}$ was assessed from surrogate pairs preserving cross-correlation, $NUCI^k_{\text{HP-SAP}}$ was linearly related to $k$ at B (Fig. 3b) and after CL (Fig. 3j) with a correlation coefficient of 0.20 and 0.24 respectively, thus suggesting that at least a part of the dependence of $NUCI^k_{\text{HP-SAP}}$ on $k$ shown in Fig. 2a and e was due to linear properties of the HP–SAP variability relation. Nevertheless, while the slope of the linear regression in Fig. 3b was coincident with that in Fig. 2a, the slope of the regression line in Fig. 3j was significantly steeper than that in Fig. 2e, thus suggesting that after CL nonlinear properties were an essential part of the relation of $NUCI^k_{\text{HP-SAP}}$ on $k$.

Fig. 4 shows baroreflex sensitivity, $\alpha$, and squared HP–SAP coherence, $K^2_{\text{HP-SAP}}$, as assessed from spontaneous HP and SAP variabilities via spectral and cross-spectral methods. Indexes are depicted as mean plus standard deviation over the entire group of subjects. Bar graphs relevant to $\alpha$ computed in the LF and HF bands (i.e. $\alpha$(LF) and $\alpha$(HF)) are depicted in Fig. 4a and b respectively. Both $\alpha$(LF)
The slope of the regression line in (e) was significantly steeper than that in (a) with \( p < 0.05 \).

Fig. 2. Individual values of \( \text{NUC}_{\text{HP-SAP}} \), computed from the original HP and SAP series, are shown as a function of the forecasting time \( k \). \( \text{NUC}_{\text{HP-SAP}} \) is calculated at B (a) and after AT (b), AT + PR (c), PR (d) and CL (e). The regression line (solid line) and the 95% confidence interval of the regression line (delimited by the dotted lines) are plotted when the regression line has slope significantly different from 0 with \( p < 0.05 \). The slope of the regression line in (e) was significantly steeper than that in (a) with \( p < 0.05 \).

4. Discussion

This study typifies for the first time the information carried by HP given SAP changes during pharmacological challenges according to two parameters: the 0-step-ahead information describing the immediate effects of SAP variations on HP and the slope of the regression line of the k-step-ahead information on the forecasting time \( k \). The main findings of the study can be summarized as follows: i) 0-step-ahead information of HP given SAP changes was significantly lower than that computed from uncoupled surrogate pairs at B and after PR and CL, while no significant difference was observed after AT and AT + PR; ii) 0-step-ahead information of HP given SAP variations was significantly lower than that computed from surrogate pairs preserving linear HP–SAP variability relation after CL, while no significant difference was observed at B, and after AT, AT + PR and PR; iii) 0-step-ahead information of HP given SAP changes significantly decreased after CL compared to B; iv) the k-step-ahead information of HP given SAP modifications was linearly related to the forecasting time at B and after CL; v) the linear relation of the k-step-ahead information of HP given SAP changes on the forecasting time was due to the HP–SAP variability relation, it was completely explained by the linear properties of the HP–SAP variability relation at B and it implied the presence of HP–SAP nonlinearities after CL; and vi) comparison with traditional indexes such as baroreflex sensitivity and squared HP–SAP coherence clearly pointed out the nonredundant information derived from the proposed analysis.

4.1. Information domain analysis of HP–SAP variability interactions supplements traditional approaches

Usually HP–SAP variability interactions are studied through time and frequency domain approaches mainly devoted to the characterization of sensitivity, latency and coupling strength of the HP–SAP variability relation (Robbe et al., 1987; Bertinieri et al., 1988; Pagani et al., 1988; Panerai et al., 1997; Cooke et al., 1999; Porta et al., 2000a; Westerhof et al., 2006; Silvani et al., 2008; Cividjian et al., 2011). When these approaches have been applied during pharmacological interventions challenging autonomic nervous system control it was found that baroreflex sensitivity significantly decreased during vagal blockade induced by AT, and significantly increased after peripheral \( \beta \)-adrenergic blockade induced by PR and central sympathetic blockade induced by CL (Parlow et al., 1995). Time delay along the pathway from SAP series to HP series, assessed as the time lag maximizing the cross-correlation function, differed during sleep stages compared to quiet wakefulness (Silvani et al., 2008) and shifted toward higher values with the progressive sympathetic activation induced by graded head-up tilt test (Westerhof et al., 2006). Coupling strength estimated as the maximum of the normalized cross-correlation function in the time domain (Westerhof et al., 2006) or via the computation of the squared coherence function (De Boer et al., 1985) significantly increased in experimental conditions challenging baroreflex control such as during orthostatic challenge (Nollo et al., 2005; Westerhof et al., 2006). Although the significance of these approaches is indisputable, as corroborated by recent findings in clinical setting (La Rovere et al., 2011), an important feature of the HP–SAP variability relation might be lost. This feature is linked to the quantification of the amount of information carried by HP that cannot be derived from SAP changes (i.e. the degree of HP unpredictability given SAP variations) (Porta et al., 2011). The novelty of the information domain approach lies in its ability to assess the strength of coupling from SAP variability to HP and \( K^2_{\text{HP-SAP}}(\text{HF}) \) compared to B.
variability, thus being closely linked to the concept of causality, disregarded by traditional measures. The magnitude of the coupling from SAP variability to HP variability was separated from that along the reverse casual direction (i.e. from HP variability to SAP variability), thus being more specifically linked to spontaneous baroreflex (Porta et al., 2011). In addition, when performed in association with surrogate data (Palus, 1997; Prichard and Theiler, 1994), the proposed approach allows the test of the null hypothesis of HP–SAP uncoupling and linear HP–SAP variability relation. Given the above mentioned conceptual differences with traditional approaches the proposed method is worth being applied to describe spontaneous baroreflex features that otherwise would remain impossible to quantify. From a more pragmatic standpoint the direct comparison of Fig. 1a with Fig. 4a and b clearly indicates the complementary information that can be derived from the proposed analysis: Indeed, baroreflex sensitivity was successful in separating AT and AT + PR from B because the magnitude of the HP–SAP variability relation vanished, while 0–step-ahead information carried by HP given SAP changes was successful in distinguishing CL from B, because the strength of the causal coupling from SAP series to HP series significantly increased. The same distinction could be obtained using the squared coherence in the HF band (Fig. 4d) but, given the linear nature of this parameter, it would have been impossible to find

**Fig. 3.** Individual values of NUC\textsuperscript{HP-SAP}, computed from surrogate pairs, are shown as a function of the forecasting time k. NUC\textsuperscript{HP-SAP} is calculated over uncoupled surrogates preserving distribution and power spectra in (a, c, e, g, and i) and over surrogates preserving cross-correlation in (b, d, f, h, and j). Surrogate pairs are built at B (a and b) and after AT (c and d), AT + PR (e and f), PR (g and h) and CL (i and j). The regression line (solid line) and the 95% confidence interval of the regression line (delimited by the dotted lines) are plotted when the regression line has slope significantly different from 0 with p < 0.05.
out the relevant contribution of nonlinear mechanisms to the increase of the magnitude of the association between HP and SAP series after CL (Fig. 1c).

4.2. Dependence of the 0-step-ahead information carried by HP given SAP changes on the state of the autonomic nervous system

When the information carried by HP given SAP changes was described according to the 0-step-ahead information parameter we found that this index was significantly smaller than that computed between uncoupled surrogates at B (i.e. the null hypothesis of uncoupling from SAP series to HP series was rejected at B). The active role of both vagal and sympathetic branches of the autonomic nervous system might be responsible for the significant coupling from SAP variability to HP variability at B. Complete vagal blockade increased the 0-step-ahead information carried by HP given SAP changes to a level that the null hypothesis of uncoupling from SAP series to HP series could not be rejected. This finding suggests that vagal activity is necessary to keep high the HP–SAP coupling and strengthens the observation that AT opens the HP–SAP closed loop along the temporal direction from SAP variability to HP variability (i.e. along baroreflex) (Parlow et al., 1995). Combined vagal and sympathetic blockade carried out by the administration of PR after AT (i.e. AT + PR) confirmed the ability of AT to uncouple HP and SAP dynamics and suggested that the reduction of the sympathetic drive induced by PR was not sufficient to facilitate vagal influences to a level capable to significantly decrease the 0-step-ahead information carried by HP given SAP variations (i.e. improving the coupling from SAP series to HP series). A similar uncoupling was obtained in cats after sino-aortic denervation opening the HP–SAP closed loop at the level of baroreflex (Di Rienzo et al., 2002, 2009). Conversely, PR and CL induce an enhancement of vagal influences by reducing the inhibitory action of the sympathetic control over vagal circuits and/or, in the case of CL, by directly increasing vagal drive (Parlow et al., 1995), we suggest that the level of vagal regulation plays a role in modulating 0-step-ahead information carried by HP given SAP changes and, consequently, the strength of the HP–SAP causal coupling. Since the 0-step-ahead information carried by HP given SAP variations decreased progressively with the sympathetic activation produced by graded head-up tilt, thus leading to a gradual increase of the strength of the causal coupling from SAP series to HP series with tilt table inclination (Porta et al., 2011), we can conclude that the degree of HP–SAP coupling increased as a result of the augmentation of both vagal and/or sympathetic activities. The positive dependence of the 0-step-ahead information of HP given SAP changes in presence of a sympathetic activation or inhibition makes clearer the complementary nature of this parameter with respect to baroreflex sensitivity and latency. Indeed, in the case of both baroreflex sensitivity and latency, opposite effects were observed in presence of sympathetic activation or inhibition with decreasing baroreflex sensitivity and increasing time shift in presence of sympathetic activation (Cooke et al., 1999; Westerhof et al., 2006; Porta et al., 2011), while the opposite behavior was observed in presence of sympathetic inhibition (Parlow et al., 1995). Comparison with surrogates preserving linear HP–SAP variability relation (i.e. cross-correlation) indicates that the significant coupling from SAP series to HP series within the same cardiac beat observed at B and after peripheral sympathetic blockade induced by PR is compatible with the presence of a linear HP–SAP variability relation. Conversely, the significant increase of the strength of the causal coupling from SAP variability to HP variability within the same cardiac beat observed after CL can be only partially explained by an increased strength of the linear HP–SAP variability interactions, thus suggesting that CL enhances nonlinearities that might be unveiled by the removal of sympathetic inhibition on vagal control and/or by the cardiac parasympathetic activation both performed at the central level.

4.3. Dependence of the rate of increase of the k-step-ahead information carried by HP given SAP changes on the state of the autonomic nervous system

When the information carried by HP given SAP changes was described by monitoring the course of k-step-ahead information over k, a significant positive relation with k is expected because the unreliability of prediction grows more and more with the temporal distance between the conditioning pattern and the value to be predicted.
(Sugihara and May, 1990; Wales, 1991; Tsionis and Elsner, 1992). As a matter of fact, a significant linear relation of the k-step-ahead information of HP given SAP variations on k was found at B and after CL with the slope of the regression line after CL significantly steeper than that at B. This linear relation is not expected only when the conditioning patterns are useless to reduce the uncertainty associated to future behaviors (e.g. when the two series are uncoupled). In the present study no significant linear relation was found over uncoupled surrogates in any experimental condition and over original data after AT and AT + PR, thus stressing that the presence of a significant HP–SAP link is a prerequisite for the observed course of the k-step-ahead information with k and that vagal control governs the loss of predictability of HP given SAP changes with k. Although the 0-step-ahead information carried by HP given SAP variations was significantly different from that assessed from uncoupled surrogates after PR, the null hypothesis of flat slope of the linear regression of k-step-ahead information on k could not be rejected. This result is the effect of a small rate of increase of the k-step-ahead information with k preventing the rejection of the null hypothesis of slope equal to 0 at the adopted significance level. It is worth stressing that the slope of the regression line of k-step-ahead information on k assessed from the original series after CL was found significantly different from that derived from surrogates preserving cross-correlation function, thus suggesting the nonlinear mechanisms unveiled by CL played a role in governing not only immediate effects of SAP variations on HP, as suggested by nonlinear interactions between respiratory centers and vagal outflow. In addition, this approach is recommended to detect nonlinearities of the HP–SAP variability interactions after CL. These results suggest that both immediate and short-term effects of SAP variations on HP are under vagal control, and corroborate the importance of vagal influences in governing the behavior of the spontaneous baroreflex in the information domain.

5. Conclusions

The study emphasizes the need to routinely apply information domain analysis when typifying baroreflex from HP and SAP spontaneous variabilities. Indeed, this analysis provides complementary information regarding to causality and nonlinearities which is ignored by more traditional approaches assessing the gain and the strength of the HP–SAP variability relation. Information carried by HP given SAP changes is primarily under vagal control. Indeed, pharmacological interventions inducing a vagal blockade or a reduction of the sympathetic inhibition on vagal circuits affect the information carried by HP given SAP changes in opposite directions. In addition, this approach is recommended to detect nonlinearities of the HP–SAP variability relation. While the HP–SAP variability relation was found inherently linear, nonlinear mechanisms were revealed by a pharmacological intervention inducing a central sympathetic blockade and, simultaneously, a central cardiac parasympathetic activation (i.e. CL), thus probably making more evident the nonlinear interactions between respiratory centers and vagal outflow. Future studies should be focused on comparison with different information domain techniques such as joint symbolic analysis (Baumert et al., 2002; Wessel et al., 2009) and mutual information (Osaka et al., 1998; Pompe et al., 1998) and on the extension of this approach to predictability-based methods (Faes et al., 2008; Riedl et al., 2010). Since in this study parasympathetic activation was accompanied by sympathetic inhibition, future studies should be focused on experimental maneuvers or pharmacological interventions, such as the administration of low dose of atropine or head down tilt, inducing a selective parasympathetic activation.

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