How to measure baroreflex sensitivity: from the cardiovascular laboratory to daily life

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Arterial baroreflex function in humans is commonly assessed through a number of laboratory tests based on quantification of the reflex responses in heart rate or blood pressure to external stimuli applied to the cardiovascular system. Evidence is available that these laboratory estimates of baroreflex sensitivity have both pathophysiological and clinical relevance. Indeed, a number of studies have shown that the sensitivity of the baroreceptor–heart rate reflex may have a prognostic value in myocardial infarction, heart failure and diabetic patients, where mortality seems to be inversely related to the sensitivity of cardiac baroreflex modulation. A deeper insight into the features of daily-life baroreflex cardiovascular control has been offered more recently by techniques based on computer analysis of spontaneous blood pressure and heart rate fluctuations. This innovative approach allows spontaneous baroreflex sensitivity to be assessed in real life conditions, with no need for external stimulation of the patient as required by the older laboratory techniques. This review will briefly survey the methods most widely used to assess baroreflex function in humans, in the laboratory and in daily life. *J Hypertens* 2000, 18:7–19 © Lippincott Williams & Wilkins.

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Introduction

The arterial baroreflex represents a mechanism of fundamental importance for blood pressure (BP) homeostasis, and its impairment may play an adverse role in several cardiovascular diseases [1–5]. An example is congestive heart failure, in which the well known increase in sympathetic activity is responsible for a reduction in baroflex sensitivity, and this reduction, in turn, may be responsible for a major impairment of baroreceptor ability to restrain sympathetic tone [6], contributing to the sympathetic activation itself and negatively affecting prognosis [8,9]. Another example is myocardial infarction, in which an impaired baroreflex modulation of cardiac function is associated with an increased mortality rate [3–5,10–13]. Finally, baroreflex abnormalities have been described early in the course of diabetes mellitus and hypertension, contributing in the former case to the occurrence of hypotensive episodes and in the latter to maintenance of the BP elevation [1–3,14]. The clinical relevance of baroreflex dysfunction is also supported by studies showing that interventions which improve baroreflex sensitivity (BRS), such as physical training [12,15] or β-adrenergic receptor blockade [1,11,16], may also beneficially influence patient's prognosis [11,12].

The above findings have stimulated a growing interest in the assessment of BRS in man, both in the laboratory and in the clinical setting. This article will provide a brief description of the methods most commonly employed to this aim, with emphasis on the exciting possibilities offered by modern approaches based on computer analysis of spontaneously occurring BP and heart rate (HR) fluctuations.

Laboratory methods for assessing baroreflex sensitivity

Several methods are available to assess the arterial baroreflex function in the laboratory (Table 1). These techniques have been successfully used to clarify the physiological role of cardiovascular modulation by the baroreflex, its alterations in disease and also how these alterations can be modified by treatment [1]. All of them require the application of an external stimulus to the subject under evaluation, and provide a 'spot' quantification of baroreflex sensitivity in standardized laboratory conditions. Pioneering methods [1] include carotid sinus massage, electrical stimulation of carotid sinus nerves, anaesthesia of carotid sinus nerves and vagi, and occlusion of common carotid arteries. Because of a number of important limitations, such as poor reproducibility, heavy interference with the neural

Table 1 Laboratory methods for assessing baroreflex sensitivity in humans

- Carotid sinus massage
- Electrical stimulation of carotid sinus nerves
- Anaesthesia of carotid sinus nerves and vagi
- Occlusion of common carotid artery
- Valsalva manoeuvre
- Head-up tilting
- Lower-body negative pressure application
- Intravenous bolus injection of vasoactive agents with no (or limited) direct effect on the heart
- Intravenous stepwise infusion of vasoactive agents
- Assessment of reflex changes in muscle sympathetic nerve activity induced by blood pressure changes following vasoactive drug infusion
- Neck chamber technique

mechanisms under evaluation, considerable invasiveness, and, in some instances, a non-negligible risk for the subject to be evaluated, the above techniques are no longer employed, and have been replaced by newer laboratory methods characterized by a much lower degree of invasiveness.

Valsalva manoeuvre

This is a widely used method to explore arterial baroreflex modulation of the heart through quantification of the tachycardia and bradycardia which occur respectively during the initial decrease and the subsequent increase in BP that follow the maintenance for 15–20 s of a constant expiratory pressure of 40 mmHg [17,18]. The advantages of this technique include the simplicity of the procedure and its non-invasiveness. However, its major limitation is that it also triggers alterations in chemoreceptor and cardiopulmonary receptor activity, which makes heart rate responses less specific. Specificity is further reduced by the concomitant stimulation of skeletal muscle receptors due to the increase in respiratory muscle tone. Finally, it requires the active cooperation of the subjects under evaluation.

Head-up tilting and lower-body negative pressure application

Assessment of the cardiovascular adjustments to head-up tilting represents an easy method to test the ability of reflex mechanisms to maintain BP homeostasis. This explains its extensive use in clinical practice [19]. A clear advantage of this method is the possibility of evaluating the baroreflex by means of a natural stimulation under a condition that is crucial to physiological baroreflex function, namely when orthostasis is responsible for pooling of blood in the lower extremities, due to the action of gravity. BRS by this technique is usually quantified by the reflex effects on heart rate and peripheral resistance, but not by reflex effects on BP, because the baroreflex is in fact aimed at minimizing the BP changes during the postural shift. The responses to head-up tilting, however, have a limited specificity, because this method also produces cardiopulmonary receptor deactivation (due to a reduction in venous return and central blood volume) and vestibular stimulation which may contribute to the cardiovascular adjustments. Vestibular stimulation can be avoided if lower-body negative pressure application rather than the head-up tilting is employed. This technique can reduce venous return in a graded, controllable and long-lasting fashion, allowing reflex effects on heart rate, vasomotor tone and a number of humoral variables to be studied. In order for the stimulus to cause a BP reduction and thus a modification of baroreceptor activity, a marked reduction in venous return is required, which means that the baroreflex is only engaged upon a background of substantial cardiopulmonary reflex engagement. Thus, this method is also not specific for arterial baroreflex assessment [1,2].

Intravenous bolus injection of a small dose of a vasoactive drug

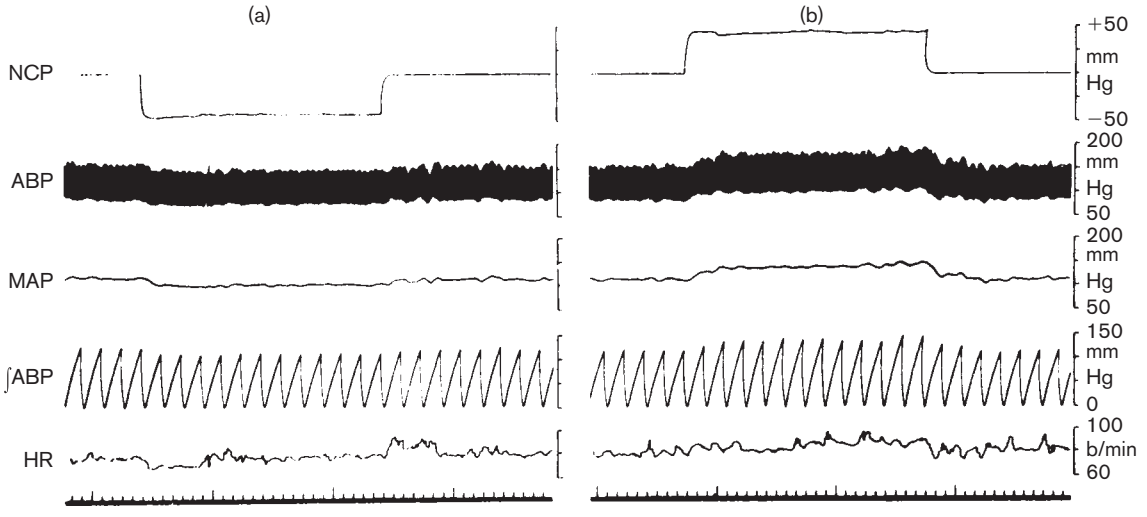
A further method for the evaluation of the baroreflex was described in the 1960s by Smyth *et al.* [1,20]. This method is based on the intravenous (i.v.) bolus injection of a small dose of a pressor agent free from any major direct effect on the heart (angiotensin II in the paper by Smyth *et al.*, and later the more vaso-selective agent phenylephrine) to increase systolic BP and reflexly lengthen pulse interval. The slope of the regression line fitting the systolic blood pressure (SBP) and pulse interval changes (usually with a lag of one beat) is taken as a measure of BRS (expressed in ms/mmHg). The same approach was later employed to assess BRS when pulse interval was shortened following a reduction in SBP induced by i.v. bolus injections of a vasodilator agent such as nitroglycerine [1,21], thus allowing investigation of the baroreflex stimulus–response curve above and below the tonic baroreceptor activity. The disadvantages of this method are that only the reflex heart rate responses can be quantified and that the responses depend almost entirely on baroreflex modulation of the vagus because they are almost entirely abolished by atropine [1]. This is more than counterbalanced by advantages such as a greater methodological simplicity compared to head-up tilting and lower-body negative pressure application and a better specificity, as shown by the disappearance of the reflex HR responses following baroreceptor denervation in animals, at least if the BP alterations induced by vasoactive agent injection do not exceed $\pm$ 20 mmHg [22]. This accounts for its persistent use in humans and for the fact that most of the current information on baroreflex impairment in disease has been obtained through this technique [3,4,9,10,23]. A later version of this technique is the 'steady-state' method proposed by Korner *et al.* [24], which is based on a prolonged infusion of either a vasopressor (e.g. phenylephrine) or a vasodepressor (e.g. sodium nitroprusside) agent to induce stepwise and sustained increases or reductions in BP, and plateau reflex changes in HR. BRS is

quantified by the ratio between the average BP (mmHg) changes during the infusion periods and the associated average reflex changes in HR (beats per min/mmHg) or R–R interval (ms/mmHg) values. A difference between the 'steady-state' and the bolus injection method is that by causing relatively long-lasting BP changes, the former may at least in part also take into account the sympathetic contribution to the baroreflex modulation of heart rate [1,25]. A drawback, however, is that the prolonged administration of vasoactive drugs may modify the tension of smooth muscle cells in the carotid and aortic walls probably more than the short-lasting bolus injection, thereby altering baroreceptor activity not only through changes in BP but also through mechanical distortion [26]. Another drawback (common to both methods) is also that all vasoactive drugs currently employed may stimulate other receptor populations (e.g. cardiopulmonary receptors) or may exert a direct stimulating effect on the sinus node [27]. Finally, compared to their bolus injection, the infusion of vasoactive drugs is also used to assess reflex changes in muscle sympathetic nerve activity, as obtained by micro-neurographic recording of the number of sympathetic bursts in the peroneal nerve, thereby allowing the sensitivity of the baroreceptor–sympathetic reflex to be quantified [28].

Neck chamber

The neck chamber device [1,29,30] consists of a rigid chamber sealed around the subject's neck, in which the air pressure is increased or reduced in a graded fashion, resulting in graded and quantifiable reductions or increases in carotid transmural pressure. The key advantage of this method is that it allows not only HR but also BP modulation by arterial baroreceptors to be investigated (Fig. 1). This has the disadvantage that only carotid baroreceptor function is assessed and that the effect of carotid baroreceptor involvement is counteracted by the aortic baroreflex, which is deactivated during the BP fall induced by carotid baroreceptor stimulation, and stimulated during the BP rise induced by the carotid baroreceptor deactivation. An additional disadvantage is that pressure changes produced within a neck chamber are not fully transmitted through the neck tissues to the carotid baroreceptors, the rate of transmission being about 80% for positive and only about 60% for negative pressure application [30,31]. This problem can only partly be solved by the use of a correction factor. Finally, the correct use of a neck chamber device requires careful training of the subject under investigation, in order to prevent possible emotional reactions to changes in pneumatic pressure around the neck that may blunt the reflex changes in HR and BP. However, with this method, important information on baroreflex control of blood pressure and systemic vascular resistance as well as on its resetting and other changes under conditions of normal and high BP has been obtained [1,32,33]. It has also been possible to demonstrate that baroreflex alterations in disease may have different effects on the control of HR and vascular resistance [1,2,33]. Finally, the only information so far available on the role of the aortic baroreflex in man has been provided by comparison of HR responses to carotid baroreceptor engagement through the neck chamber method and to overall arterial baroreceptor engagement by the vasoactive drug method [1,2].

Fig. 1



Blood pressure and heart rate effects of a 2 min application of negative (a) and positive pneumatic pressure (b) within a neck chamber device. NCP, neck chamber pressure; ABP, arterial blood pressure recorded through a catheter in the radial artery; MAP, mean arterial pressure; ∫ABP, BP values integrated every 10 s; HR, heart rate. Reproduced from [30], with permission.

The advantages and disadvantages of all the above mentioned laboratory methods are summarized in Table 2 [34,35].

Modern techniques for the analysis of spontaneous baroreflex modulation of heart rate

An important step forward in the investigation of the arterial baroreflex in humans has been the development of techniques that analyse the sensitivity of 'spontaneous' baroreflex control of heart rate [34,36]. These techniques do not require any external intervention on the subject under evaluation. Moreover, they can be used not only to assess BRS in standardized laboratory conditions, but also to investigate the dynamic features of baroreflex modulation of heart rate in daily life [34,36,37]. These methods, which are listed in Table 3, are all based on the combined computer analysis of spontaneous blood pressure and heart rate fluctuations. This justifies use of the term 'spontaneous baroreflex' function when referring to the data thus obtained [38]. Hereafter, the main features of the most commonly employed techniques for spontaneous baroreflex analysis will be discussed, namely the 'sequence technique', the spectral method based on the calculation of the 'α coefficient', and the method based on mathematical modelling, through auto-regressive and moving average (ARMA) techniques, of the mutual interactions between SBP and R–R interval which occur physiologically in a closed-loop condition.

Table 2 Advantages and limitations of currently employed laboratory methods for assessing baroreflex sensitivity in humans

Advantages
- Assessment of baroreflex function under standardized and controlled conditions
- Information of proved physiological and clinical value provided

Limitations
- Data collected in an artificial and at times stressful laboratory environment
- Only spot quantifications of baroreflex sensitivity obtained
- No information on daily-life behavioural modulation of baroreflex sensitivity
- Most stimuli are not specific for the baroreflex
- Non-physiological nature of laboratory stimuli (baroreflex function explored over a much wider range of stimulus intensities than that associated with spontaneous blood pressure fluctuations)
- A closed-loop mechanism such as the baroreflex assessed in a quasi-open loop condition (i.e. by assuming that the SBP effects on R–R interval are not simultaneously accompanied by effects of R–R interval on SBP)
- Limited reproducibility of the responses to most laboratory methods

SBP, systolic blood pressure

Table 3 Methods for assessing spontaneous baroreflex function

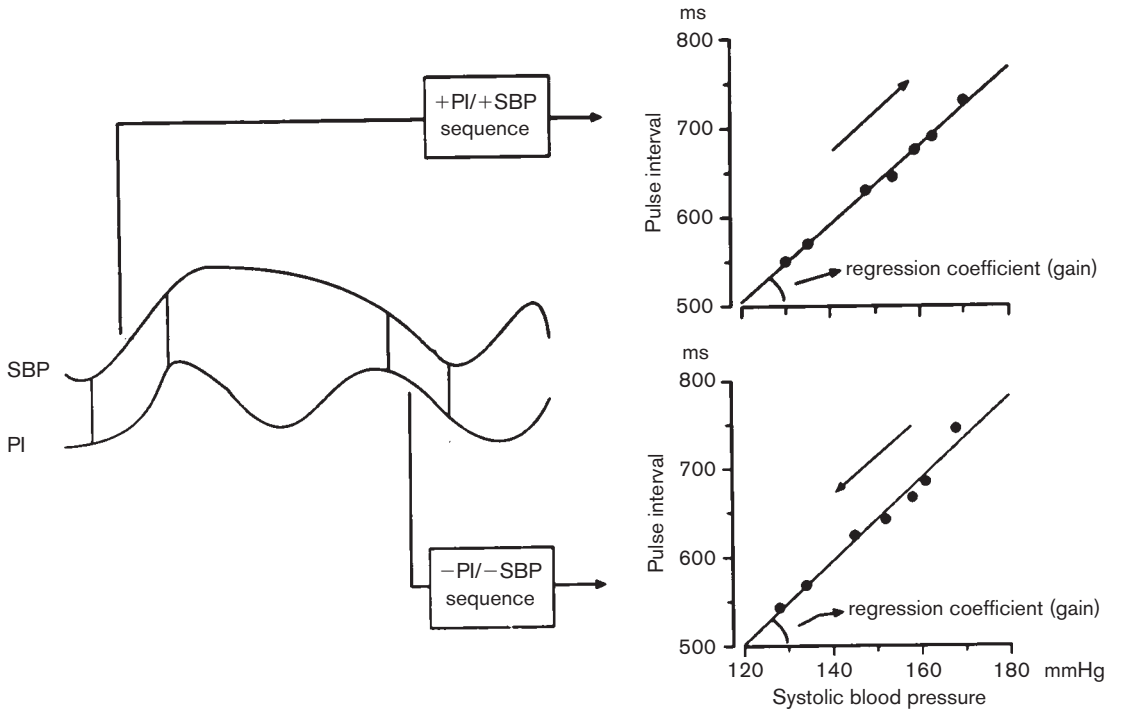
- Sequence technique (sequences of beats where spontaneous SBP changes are coupled with baroreflex-mediated R–R interval changes)
- R–R interval – SBP cross-correlation
- Modulus of R–R interval – SBP transfer function at 0.1 Hz
- Squared ratio of R–R interval/SBP spectral powers at 0.1 and 0.3 Hz (α coefficient)
- Closed-loop R–R interval–SBP transfer function (ARMA modelling)
- Statistical dependence of R–R interval on SBP fluctuations

SBP, systolic blood pressure; ARMA, auto-regressive moving average.

The sequence method

The 'sequence' method is based on the computer identification in the time domain of spontaneously occurring sequences of four or more consecutive beats characterized by either a progressive rise in systolic blood pressure and lengthening in R–R interval (+RR/+SBP sequences) or by a progressive decrease in systolic blood pressure and shortening in R–R interval (–RR/–SBP sequences) [16,39–44] (Fig. 2). The slope of the regression line between systolic blood pressure and R–R interval changes is taken as an index of the sensitivity of arterial baroreflex modulation of heart rate (BRS), as with the laboratory method based on i.v. injection of vasoactive drugs. Among the special features of this technique is that the separate identification of sequences characterized by increases or reductions in SBP allows the effects of spontaneously occurring baroreceptor stimulation and deactivation to be separately assessed. Secondly, the sequence method is based on relatively strict threshold criteria because the sequences that are considered are only those lasting at least four beats and having consecutive SBP and R–R interval changes equal to or greater than 1 mmHg and 5 ms, respectively. This gives the assessment of BRS a remarkable specificity. Experimental demonstration of this specificity, i.e. the demonstration that R–R interval changes observed in each sequence in response to the spontaneous changes in SBP are actually dependent on the baroreflex, has been performed in conscious cats, in which BP and HR were continuously recorded for 3–4 h both before and after sino-aortic denervation (SAD) [40]. In intact animals, 100 sequences of either the +RR/+SBP or the −RR/−SBP type were identified, whereas after SAD the number of both sequences became negligible. The demonstration of the specificity of the sequence technique has also been obtained by showing that the BP and R–R interval coupling seen in the spontaneous sequences identified from biological data is markedly different from their chance coupling obtained by using an isospectral and isodistribution surrogate data set [45]. Thirdly, the average BRS obtained by the sequence method over periods of standardized behaviour and of sufficient duration (at least 10–15 min) is highly reproducible [46] (Omboni *et al.*, unpublished observations). Finally, the sequence method largely reflects baroreflex control of cardiac vagal drive [38], because the length of most sequences is usually shorter than six beats [47,48] and because they almost disappear following atropine injection.

Fig. 2



The method for spontaneous baroreflex analysis based on assessment of hypertension/bradycardia and hypotension/tachycardia sequences. In the left panel, a schematic drawing exemplifies changes in systolic blood pressure (SBP) and pulse interval (PI; the reciprocal of heart rate) as a function of time (on the horizontal axis). The corresponding regression lines between changes in SBP and changes in PI are also shown in the right hand panels. Reproduced from [34], with permission.

Studies comparing the spontaneous sequence and the drug injection techniques

The sequence technique has been compared in individual subjects with laboratory methods based on i.v. injection of phenylephrine or nitroglycerine [34,38,49]. In most cases, high correlation coefficients between the average estimates of BRS obtained with the two techniques were found. The absolute figures of BRS quantified by the two methods were not super-imposable, however, presumably because the aspects of the baroreflex influences on the sinus node they address are somewhat different [34,36,38,45]. This is not surprising because, for example, as mentioned above, vasoactive drugs can induce changes in the mechanical properties of the arterial wall where baroreceptors are located, which may result in a greater and somewhat less physiological stimulus for reflex changes in heart rate at any given BP alteration [49,50]. Furthermore, the drug injection method usually allows relatively large BP changes to be produced and the baroreflex thus to be tested not only in the linear portion of its stimulus–response curve but also in the portions where baroreceptor activity approaches saturation and threshold, and BRS is smaller. Conversely, the blood pressure changes characterizing a sequence are usually small, which means that this method in most instances does not provide a 'full-range' analysis of BRS [38]. Differences between the results obtained by these two methods for assessing the baroreflex should thus be expected and these techniques should be regarded not as alternatives but as complementary approaches to evaluate baroreflex function. On the background of these considerations, it is by no means surprising that the best agreement between the sequence and the vasoactive drug bolus injection methods has been obtained by comparing the spontaneous baroreflex slope with the slope computed by (1) considering both the reflex R–R interval lengthening in response to SBP increases induced by phenylephrine and the reflex R–R interval shortening in response to SBP reductions induced by nitroglycerine, and (2) drawing the tangent to the sigmoidal baroreflex curve so obtained at a point corresponding to the subject's resting BP level. In subjects included in such a study, BRS computed at that point was 23.9 ms/mmHg while with the sequence method it was on average 25.0 ms/mmHg, with a correlation coefficient between the two of 0.96 ($P < 0.001$) [38].

Results of the sequence technique in daily life

The results provided by studies where the sequence technique has been employed have documented the

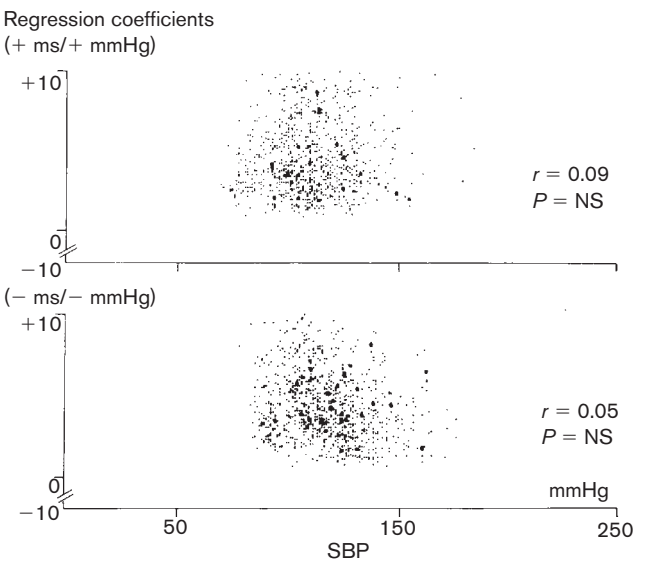
high degree of variability which physiologically characterizes BRS in different behavioural conditions (Fig. 3) [16,38–44,46]. Variations in BRS are even more pronounced when the sleep–wakefulness cycle is taken into account. The typical patterns of spontaneous baroreflex modulation of HR over 24 h obtained in a group of young and a group of elderly subjects is shown in Figure 4. Besides the occurrence of a minute-to-minute variability, BRS in young individuals showed a clear-cut difference between the day and night, with lowest values during the day and highest values during the night. In elderly subjects, BRS was not only on average smaller than in young individuals over the 24 h period, but also displayed a loss of the physiological day–night difference. In part this may reflect a reduction in the degree of daytime physical activity with ageing. It may also reflect, however, an impairment of various aspects of daily-life baroreflex HR modulation in elderly subjects [42].

Recent progress with the sequence technique

The sequence technique also offers the quantification of the number of times in daily life the baroreflex is effective in overcoming the non-baroreflex influences that regulate the sinus node (central neural influences, humoral factors, respiration, etc). This was assessed by calculating the ratio between the number of +RR/+SBP or −RR/−SBP sequences and the total number of SBP ramp-like changes, in order to derive the so-called baroreflex effectiveness index (BEI). In a preliminary application of this approach to 24 h blood pressure recordings, it was found that only a limited fraction of SBP ramp changes occurring over the 24 h are coupled with baroreflex-mediated linear R–R interval changes, suggesting that most of the time non-baroreflex influences prevail over baroreflex ones. This also suggests that the number of times the baroreflex makes its inhibitory influences on the sinus node manifest may be taken as another measure of its effectiveness in controlling circulation [51].

From a methodological point of view, the sequence method requires beat-to-beat monitoring of SBP coupled with an ECG to obtain a precise assessment of the R–R interval–SBP relationships. If computer scanning of the SBP signal is done at a sufficiently high sampling frequency (> 168 Hz), and if the BP waveform undergoes a parabolic interpolation of its peak, R–R interval can be indirectly estimated from the BP signal (by quantifying pulse interval, i.e. the interval between consecutive systolic peaks) with a degree of accuracy which is in general comparable to that of R–R interval detection from an ECG [52,53]. A further simplification of the technical requirements for application of the sequence technique to humans is also offered by the continuous and accurate SBP recording both at rest and in ambulatory conditions that can now be obtained from non-invasive devices [54–57].

Fig. 3

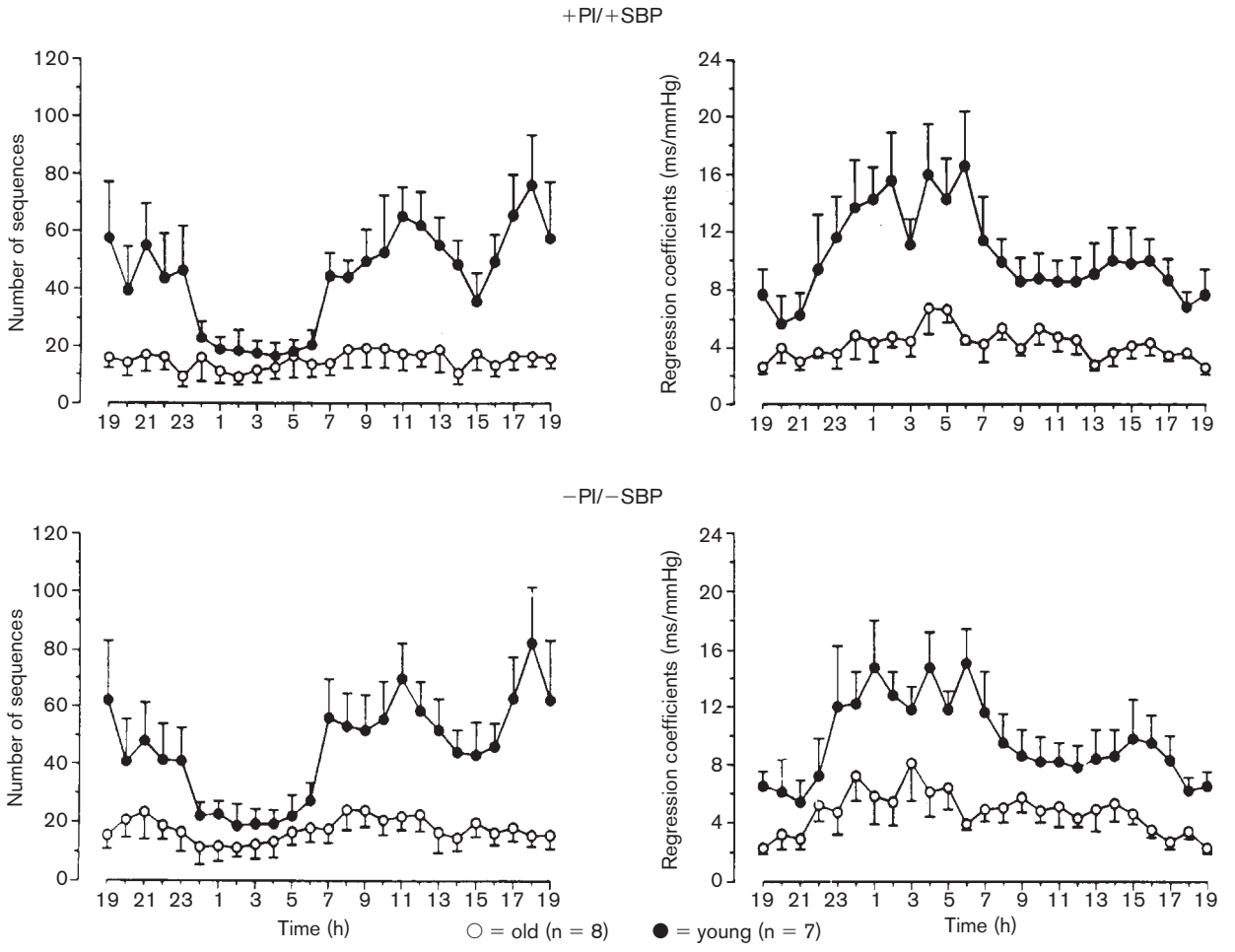


24 h distribution of the regression coefficients of four-beat long sequences (an index of baroreflex gain) as a function of systolic blood pressure (SBP) in a representative subject. Data are shown as individual sequence slopes separately for hypertension/bradycardia (+ms/+mmHg) and for hypotension/tachycardia (−ms/−mmHg) sequences. *r* values refer to correlation coefficients between sequence slope and SBP values.

The spectral technique (α coefficient)

Assessment of spontaneous BRS by the spectral technique [58–61] is based on (1) sub-division of the recorded BP and R–R interval signals into short segments ranging from 128 to 1024 beats, (2) quantification for each segment, by either the fast Fourier transform (FFT) or auto-regressive modeling, of the R–R interval and SBP spectral powers in the frequency regions around 0.1 Hz (the so-called 'low frequency', LF) and at the respiratory frequency ('high frequency', HF: around 0.2 and 0.3 Hz), where these signals usually display a high coherence (> 0.5), i.e. where the oscillations in R–R interval and SBP are linearly related, and (3) calculation of the gain of the transfer function between SB and R–R interval changes [58,59], or more simply, the squared root of the ratio of R–R interval and SBP powers, referred to as the 'α coefficient', in the above frequency regions (Fig. 5) [60,61]. This relies on the assumption that the ratio between R–R interval and SBP powers in the frequency regions where they are coherent is a reflection of baroreflex function. The specificity of this spectral index of BRS has also been assessed in conscious cats in which continuous BP and HR recordings were obtained before and after SAD [62,63]. As expected, arterial baroreceptor denervation

Fig. 4

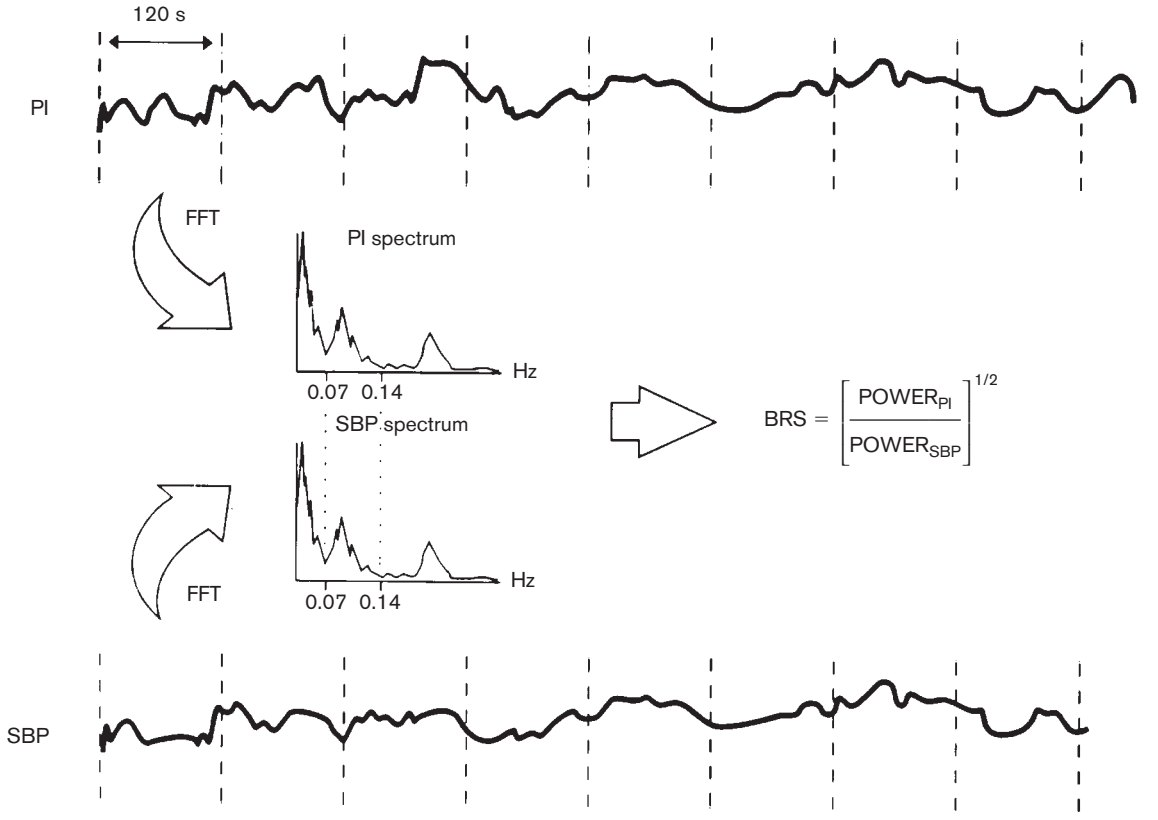


Number of hypertension/bradycardia and hypotension/tachycardia sequences (left panels) and sequence regression coefficients (right panels). Data are shown as average hourly values (± SE) for a group of young and a group of elderly individuals. Reproduced from [42], with permission. SBP, systolic blood pressure; PI, pulse interval.

induced a reduction in overall R–R interval variance which was particularly evident around 0.1 and 0.3 Hz. SBP spectral powers, conversely, changed differently in different frequency regions, i.e. SBP overall variance and the power of its very low frequency components increased, whereas the SBP power around 0.1 Hz markedly decreased, and the power around the respiratory frequency did not change. These modifications in SBP and R–R interval powers were accompanied by a significant reduction of the α coefficient computed both for the 0.1 Hz (αLF) and the HF (αHF) regions. This validates the α coefficient as a measure of BRS in the frequency domain. Additional evidence supporting the assessment of BRS through calculation of the α coefficient has been obtained by demonstrating a relatively close correlation between α coefficient values and the estimates of BRS obtained by i.v. bolus injection of phenylephrine or nitroglycerine [49,61].

The sequence technique and the α coefficient have different features, however. First, when assessing the squared ratio of R–R interval to SBP powers, the phase relationship between these variables is frequently not taken into account. Thus it cannot always be taken for granted that the changes in R–R interval associated with changes in SBP powers included in the calculation of the α coefficient are causally related. Second, the α coefficient is commonly computed by taking a high (> 0.5) R–R interval/SBP coherence value as a marker of RR/SBP coupling by the baroreflex. We have shown, however, that after SAD the number of signal segments with high (> 0.5) coherence between R–R interval and SBP powers was markedly reduced in the LF region but not in the HF one. This indicates that in the HF band the coupling between R–R interval and SBP may have a non-baroreflex origin, i.e. that concordant fluctuations in SBP and R–R interval can be produced by

Fig. 5



Schematic drawing illustrating how baroreflex sensitivity can be assessed in the frequency domain by calculation of the α coefficient. BRS, baroreflex sensitivity; SBP, systolic blood pressure; PI, pulse interval; FFT, fast Fourier transform. Reproduced from [34], with permission.

central and peripheral mechanisms (respiration, central influences, hormones, etc) by which cardiac and vascular targets are modulated in a qualitatively unidirectional fashion [63]. An indiscriminate use of coherence data throughout the LF and HF regions to identify the occurrence of R–R interval–SBP coupling by the arterial baroreflex may thus lead to results that are not all related to the baroreflex.

Comparison of the sequence and the α coefficient methods

The methods for spontaneous baroreflex analysis share the advantages of providing a reproducible estimate of the average BRS that occurs in a given condition. The sequence technique in addition has the advantage of providing information on the minute-to-minute variability of the effectiveness of baroreflex modulation of heart rate, because while calculation of the α coefficient requires a time window of no less than 128 or 256 beats (see below), the BRS values obtained by the sequence technique reflect baroreflex function even during a few seconds.

The sequence and the spectral techniques are characterized by other distinct features. The spectral technique, for example, focuses on SBP and PI rhythmic oscillations limited to relatively narrow frequency regions, which means that the α coefficients that can be derived from this approach reflect the baroreflex ability to modulate the sinus node only at specific frequencies [64,65] thus offering the possibility to separately assess the sympathetic and parasympathetic contribution to reflex heart rate modulation. On the other hand, the R–R interval–SBP sequence technique focuses on BP and R–R interval changes that may have a wider frequency content and thus the BRS estimation obtained by the sequence slope is a sort of 'comprehensive' index of the baroreflex control of the heart rate averaged for several frequencies. This is physiologically relevant because in cats SAD causes marked alterations of BP and HR oscillations over a wide range of frequencies (e.g. also below the LF region), which means that the baroreflex modulation of the sinus node is wider than that explored by the αLF and αHF coefficients. Moreover, the R–R interval–SBP sequences occur unevenly over time, as they are related to daily-life behaviours. Such an irregular occurrence does not prevent a suitable estimate of the day and night BRS profile because the number of sequences

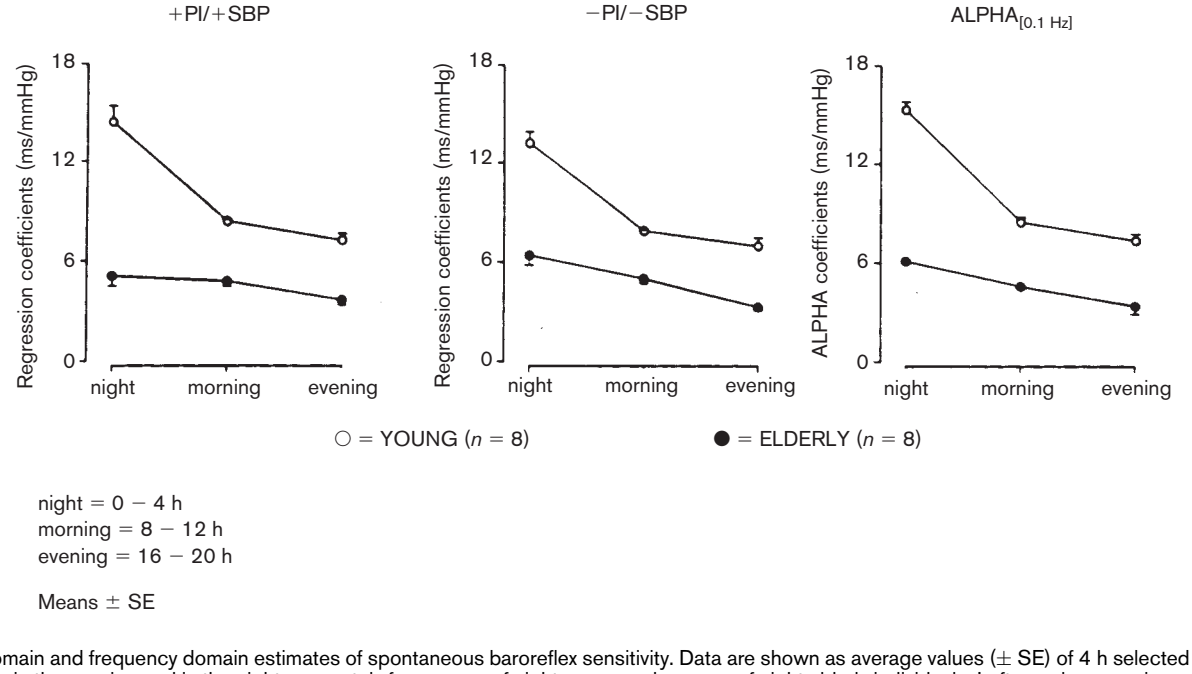
observed over each hour of a 24 h blood pressure recording is high enough to obtain a large hourly database. It may not be large enough to characterize average BRS over shorter time intervals, however. This can more suitably be obtained by α coefficients, because at the respiratory frequency and around 0.1 Hz SBP oscillations occur regularly even when short recording periods are considered [42]. A final difference is that, as mentioned above, the sequence method offers a separate assessment of the reflex R–R interval changes induced by baroreceptor stimulation (+RR/+SBP sequences) and baroreceptor deactivation (−RR/−SBP sequences), which may be of interest in clinical conditions such as the obstructive sleep apnoea syndrome [43]. This is not possible with the spectral technique which groups together the reflex influences of BP changes leading to baroreceptor stimulation and deactivation.

Despite the above differences, the quantification of the BRS parameters based on computation of α coefficients and on the sequence method appears to be qualitatively and quantitatively similar when averaged over a time window of at least a few minutes. This is the case when assessing the effects of SAD in animals, after which both time domain and frequency domain indices of baroreflex sensitivity are markedly reduced [36,40,63]. This is also the case when comparing average BRS data obtained over 24 h or over a 15 min recording in a clinical environment, as observed in subjects of different age [42,44] (Fig. 6) and in diabetic patients with or without laboratory evidence of autonomic dysfunction [66].

Auto-regressive, moving average modelling techniques

The techniques for signal analysis mentioned above derive BRS estimates from the direct quantification of the effects of SBP changes on R–R interval. Other more complex methods have been proposed, however, which evaluate baroreflex function by mathematical modelling of cardiovascular regulatory mechanisms. In this instance, biological signals are used to identify the parameters of a pre-selected mathematical model of the baroreflex. This is aimed at accounting for the inherent complexity of the interactions between BP and R–R interval which physiologically occur in a 'closed-loop' condition, i.e. in a condition where not only changes in BP induce reflex changes in R–R interval (BP → R–R feedback), but also where, at the same time, changes in R–R interval are responsible for changes in BP mediated through changes in cardiac output (R–R → BP feed-forward). The picture is even more complex if we consider that BP values at any given time point, t, also depend on BP values at the preceding times, $t - i$, this being the case also for HR values. Finally, BP and HR values are simultaneously affected by respiratory activity as well as by a number of other external or internal influences considered as 'noise'

Fig. 6



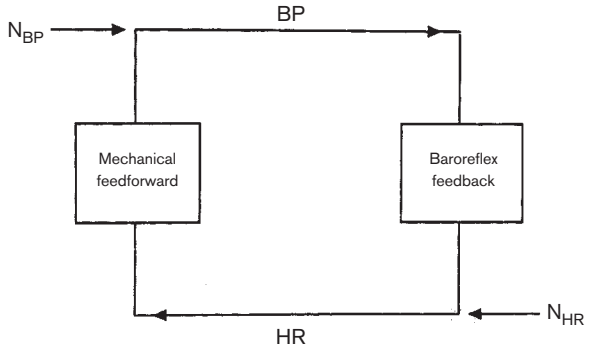
Time domain and frequency domain estimates of spontaneous baroreflex sensitivity. Data are shown as average values (± SE) of 4 h selected in the morning, in the evening and in the night, separately for a group of eight young and a group of eight elderly individuals. Left panel: regression coefficients of hypertension/bradycardia sequences (+PI/+SBP); middle panel: regression coefficients of hypotension/tachycardia sequences (−PI/−SBP); right panel: α coefficients computed in the frequency region around 0.1 Hz. Reproduced from [42], with permission.

factors, i.e. factors not specifically accounted for in the model (Fig. 7) [67]. Once a mathematical model has been analytically defined in order to take all the considered mechanisms into account, the transfer function (and therefore the gain), the delay and the time constants that characterize baroreflex control on cardiovascular variables can be derived by proper handling of the equations which describe the model. The accuracy of these estimates depends on how well the unavoidable simplifications inherent in the mathematical model fit the physiological complexity of cardiovascular control mechanisms.

An example of such models is represented by the method based on a closed-loop analysis that simultaneously considers the BP → R–R interval feedback, the R–R interval → BP feed-forward, the effects of previous BP or R–R interval values on the values actually measured and the concomitant effects of respiration on both BP and R–R interval variability, by a trivariate ARMA approach applied to the analysis of continuous BP, R–R interval and respiratory activity recordings. A pre-requisite for such an analysis, the mathematical representation of which is shown in Figure 8, has been the use of paced random-interval breathing, which was necessary to induce random perturbations in BP through changes in respiratory mechanics and thus obtain random variations in the input signal to arterial baroreceptors (i.e. the BP changes) [47,68].

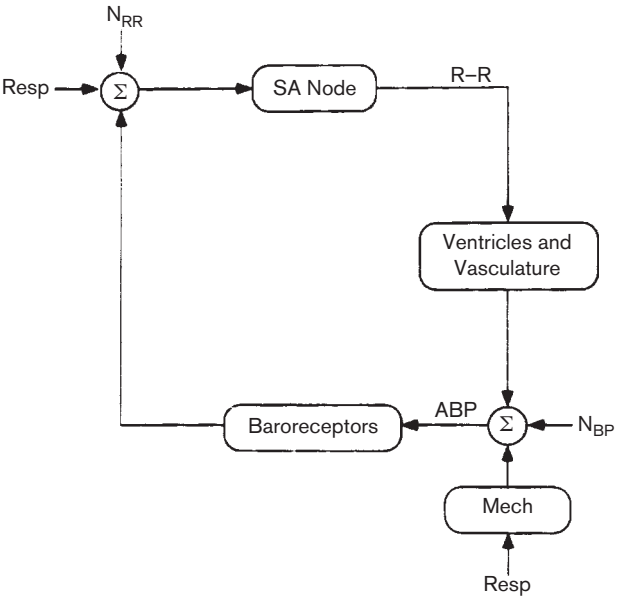
In the formula, the coefficient a_i represents the auto-regressive parameter of R–R interval, which quantifies the effect of previous R–R values on the R–R value occurring at time t, while the corresponding moving-average parameter vectors b_i and c_i quantify the effects on R–R interval of respiration (respiratory sinus arrhythmia) and of arterial (A) BP (arterial baroreflex), respectively. As mentioned above, these parameters have been used to calculate the transfer functions and thus the gain, the phase shift and the coherence values that characterize the effects of respiration on R–R interval or on BP, the effects of BP on R–R interval and the effects of R–R interval on BP. The BRS values obtained by this approach (quantified by the gain of the BP → R–R interval transfer function) were found to be significantly lower than those provided by the drug bolus injection technique, in spite of a significant between-method correlation [67]. This confirms, retrospectively, the participation of more complex factors,

Fig. 7



Schematic drawing which illustrates the reciprocal interactions between blood pressure (BP) and heart rate (HR) fluctuations, including the reflex effects of BP on HR (baroreflex feedback) and the mechanical effects of HR on BP (mechanical feed-forward). N_{BP} and N_{HR} refer to 'noise' factors (i.e. factors independent from HR and BP) respectively acting on BP and HR. Reproduced from [34], with permission.

Fig. 8



$$RR(t) = N_{RR} + \sum_{i=1}^{j} a_i\, RR(t\text{-}i) + \sum_{i=S_r}^{fr} b_i\, Resp(t\text{-}i) + \sum_{i=S_A}^{fa} c_i\, ABP(t\text{-}i)$$

Schematic drawing illustrating the interactions between respiration (Resp) and R–R interval, between R–R interval and arterial blood pressure (ABP), between ABP and R–R interval through the baroreflex, and between Resp and ABP. The scheme illustrates a simple closed-loop feedback and control model of arterial blood pressure (ABP) and heart rate regulation. Respiration (Resp) is modelled to affect R–R interval and ABP by both central modulation of autonomic activity and by mechanical coupling (Mech) of respiratory activity to ABP. Additional 'noise' sources, N_{RR} and N_{BP}, are included to account for R–R interval and BP fluctuations not accounted for by measured signals. SA node, sino-atrial node. At the bottom, the auto-regressive moving average (ARMA) equation used to describe the interactions between ABP, Resp and R–R interval is shown (see text). Reproduced from [67], with permission.

such as the R–R interval–SBP feed-forward component, in calculation of the BRS obtained by laboratory methods or even by the sequence and the α coefficient methods.

Spontaneous baroreflex sensitivity in health and disease

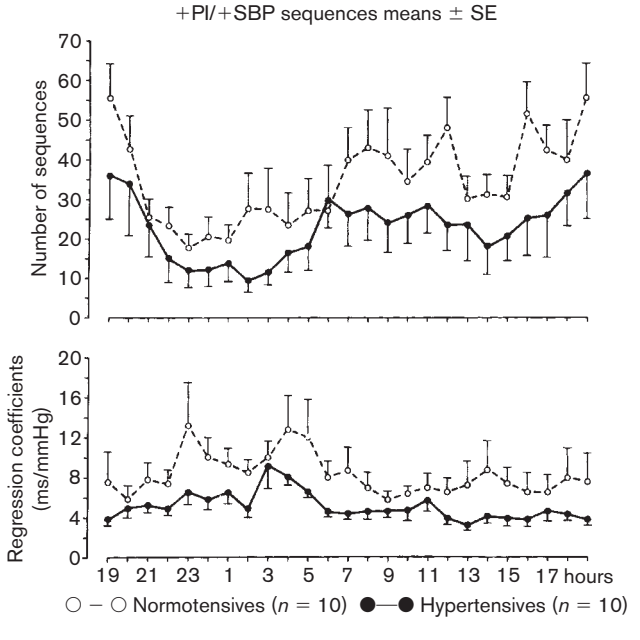
Data obtained by either time and frequency domain methods for assessing spontaneous BRS have provided a large body of information on daily-life baroreflex function in health and disease. This information comprises of detailed evidence on the fast and marked changes in BRS associated with changes in behavioural conditions as well as with particular situations such as general anaesthesia [34,69]. It also includes description of the day and night reduction in BRS in hypertensive or aged subjects as compared to normotensive or young individuals [41,42] (Figs 4 and 9)), together with data on the impairment of BRS associated with cigarette smoking (an impairment which escapes recognition when traditional laboratory methods are employed) [70] and on the striking baroreflex dysfunction typical of patients with primary autonomic failure [71] or of patients with obstructive sleep apnoea syndrome [43].

Assessment of spontaneous BRS has also been performed in diabetic patients with or without clinical evidence of autonomic dysfunction, as documented by classical laboratory tests. The results have shown that both time and frequency domain methods can identify an impairment of baroreflex control of HR at a time when traditional testing still yields normal results [66] (Fig. 10), emphasizing the superiority of these techniques over traditional laboratory procedures in the early detection of autonomic abnormalities that may carry an increased risk of morbidity and mortality [44,72].

Conclusions

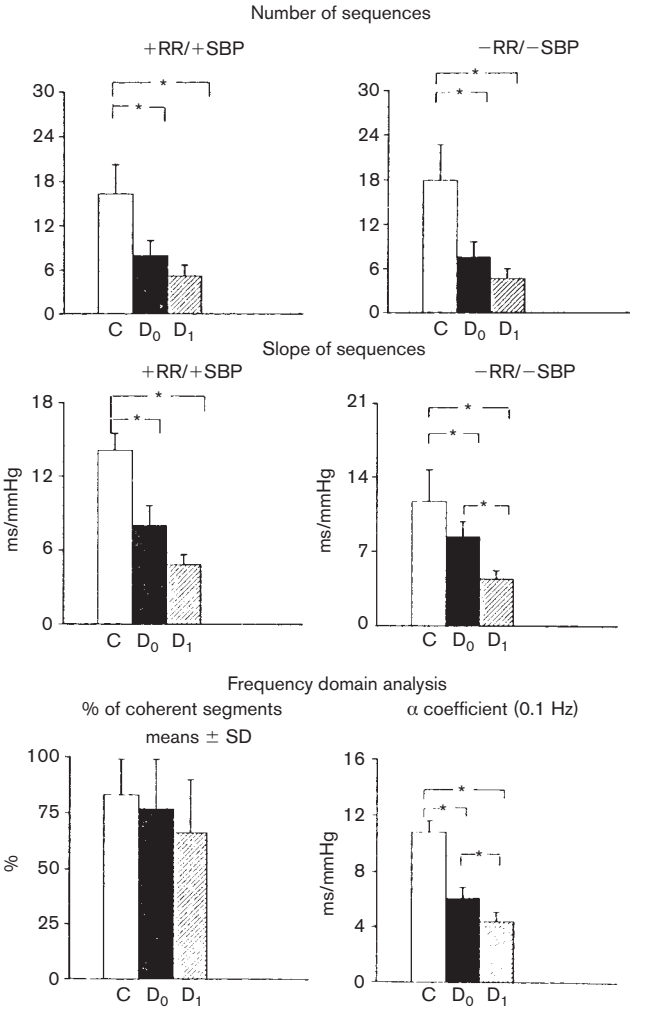
This review article has summarized some of the pros and cons of different methods for assessing BRS in humans. Laboratory methods have largely contributed to our present knowledge of human arterial baroreflex

Fig. 9



Hourly number of hypertension/bradycardia sequences (+PI/+SBP) (upper panel) and hourly values of the regression coefficients of these sequences (lower panel). Data are shown as average values (± SE) for a group of 10 normotensive and a group of 10 hypertensive individuals. Reproduced from [41], with permission. SBP, systolic blood pressure; PI, pulse interval.

Fig. 10



Time and frequency domain estimates of the baroreceptor–heart rate reflex in control subjects (C) ($n = 24$), diabetic patients without (D_0) ($n = 20$) or with (D_1) ($n = 32$) autonomic dysfunction at classical laboratory tests . $^*P < 0.05$. Reproduced from [66], with permission. SBP, systolic blood pressure;

function. Modern techniques for spontaneous baroreflex analysis, however, have more recently provided us with a deeper insight into how this important mechanism for cardiovascular regulation works in daily life [37,41,59, 61,67,73].

Whether this richer amount of information may further improve the clinical evaluation of patients, in whom a baroreflex impairment as assessed by traditional methods has been shown to negatively affect prognosis, remains to be seen in large-scale longitudinal studies.

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