Applicability of new techniques in the assessment of arterial baroreflex sensitivity in the elderly: a comparison with established pharmacological methods

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(Received 10 March/30 October 1997; accepted 10 November 1997)

INTRODUCTION

The estimation of arterial baroreceptor–cardiac reflex sensitivity (BRS) has for over 25 years formed an important part of the study of cardiovascular disease and particularly hypertension since the first descriptions of a reproducible method by the Oxford group [1, 2]. The method assumes a static linear relationship between stimulus (change in systolic blood pressure) and response [change in pulse interval (PI)] and has changed little since its first description, although newer practical techniques such as the non-invasive registration of beat-to-beat blood pressure (BP) using the Finapres device have been introduced [3]. However, more recently, two alternative analyses have been developed which obviate the need for drug-induced BP disturbances in the assessment of BRS. The first consists of the computerized scanning of long recordings of simultaneous BP and PI for sequences in which BP and PI are either rising (+BP/+PI) or falling (−BP/−PI) in parallel for at least three beats, representing the spontaneous activity of the arterial baroreflex at rest [4–6]. The second method consists of the frequency domain analysis of the variability in BP and PI by power spectral analysis [7]. This enables the linkage or cross-spectrum between the BP and PI signals to be quantified in terms of amplitude or gain, phase (the time shifts between the two signals) and coherence. Previous studies have suggested that in humans coherence is acceptable in two frequency regions usually referred to as low frequency (LF 0.05–0.15 Hz, sometimes called the mid-frequency) and high frequency (HF 0.20–0.35 Hz) [8–10]. Recent work has summarized the interrelation of BP and PI in the frequency domain in terms of the indices aLF and aHF and their mean a, descriptive of the overall activity of the baroreflex [9, 10].

Given our understanding of the arterial baroreflex based on existing pharmacological methods, the
question arises as to the comparability of results obtained by the two newer methods. This issue has been addressed at least in part in a number of studies. A recent study in a small group of young normotensive subjects [11] comparing sequence analysis with pharmacological methods (with phenylephrine and sodium nitroprusside injection) found considerable agreement between BRS assessed by these two time domain techniques. Two other studies [8, 9] have described a close relation between BRS from spectral analysis and that derived from the phenylephrine injection method. Two recent studies [6, 12] compared BRS values from sequence and spectral analysis and both found significant correlations between sequence BRS and αLF, αHF and the overall index z. In contrast, another recent study [13] in a large group of young normotensive and borderline hypertensive subjects found substantial systematic bias between BRS results obtained from the phenylephrine method compared with sequence and spectral estimates, with consequent poor agreement between the respective methods. However, there is only one published study which has included a comparison of the two newer methods in a small group of older subjects [14], which described the results as 'super-imposable' without attempting to quantify the level of agreement. Thus the purpose of the present study was to examine the comparability of results obtained by the traditional drug-based methods with those from the newer sequence and spectral techniques in a group of elderly subjects across a sufficiently wide range of BPs to include both hypertensive and normotensive subjects, and the present study is the first to simultaneously examine pharmacological, sequence and spectral methods in this age group.

METHODS

We studied 22 elderly (aged over 60 years) subjects of age 68 ±1 years (mean ±SEM; range 60–75 years) who were non-smokers and free from major disease. Hypertensive subjects were recruited from among those referred to a large teaching hospital for assessment and management of their hypertension, and normotensive subjects from the spouses and friends of the hypertensive subjects or from the respondents to advertisements in the local press. All subjects were active and ambulant and living independently in the community. None was taking any medication with known cardiovascular effects, and none of the hypertensive subjects had previously received any antihypertensive medication. Subjects with a history or clinical evidence of other cardiovascular disease including atrial fibrillation, other disorders associated with autonomic dysfunction or other major illness were excluded. No subject in the normotensive group had any history of hypertension or pre-eclampsia. All subjects gave written informed consent to participate in the study, which received local ethical committee approval and was conducted in accordance with the Declaration of Helsinki.

Sustained clinic BP was characterized from a total of nine readings, averaged from three readings taken on each of three visits over a 6 week run-in period. BP was measured with the subject supine after a minimum 5 min rest using a conventional sphygmomanometer (diastolic Korotkoff phase V) and a cuff of appropriate size. Subjects with an average sustained clinic BP of systolic BP ≥ 160 mmHg and/or diastolic BP ≥ 90 mmHg were regarded as hypertensive, and thus 11 subjects (8 female, 3 male) were classified as hypertensive and 11 (4 female, 7 male) as normotensive.

Laboratory studies

All subjects attended the research laboratory for a morning session in the post-absorptive state, having refrained from alcohol and caffeine for at least 12 h. Subjects were light clothing and the laboratory temperature was thermostatically controlled to 20–22°C. Subjects rested supine for a minimum of 30 min after the insertion of a cannula into a dorsal hand vein, and were fitted with chest leads for recording of the continuous surface ECG (model CR7; Cardiac Recorders Ltd, London, U.K.). The appropriate-sized finger cuff of the Finapres 2300 non-invasive beat-to-beat BP recording device (Ohmeda Monitoring Systems, Englewood, Colorado, U.S.A.) was fitted to the middle finger of the non-dominant hand, which rested throughout on an adjustable support at the level of the heart.

After achievement of a satisfactory BP signal from the Finapres and the stabilization of BP at the same level for at least 10 min, subjects went through a three-stage study protocol. During the first stage, three periods of continuous BP and PI were recorded, each of 5 min duration, for sequence and spectral analysis, with the subject resting supine and maintaining a respiratory rate above 12 breaths/min, and without any external stimulation such as talking. During each of these periods the servo self-adjust mechanism of the Finapres was disabled, but the device was recalibrated between each recording period. If there was any substantial discrepancy between BP at the end of any recording period and the BP after recalibration, that record was discarded and a repeat recording made after a further stable period, although in practical terms this was only required on a very small number of occasions [3, 15].

The two subsequent stages consisted of BRS testing by the established pharmacological methods:

1. The BP and PI response to phenylephrine injection [1]. An initial bolus dose of 50 µg of phenylephrine was progressively increased in 50 µg steps as necessary (up to a maximum of 200 µg) to achieve a peak BP rise of 20–40 mmHg, and the effective dose
was repeated to obtain a minimum of three adequate responses. Bolus injections of 0.9% saline were interspersed at random between the drug injections and the subject was blinded to the nature of each injection.

2. The BP and PI response to a graded sodium nitroprusside infusion [16–18]. The infusion was commenced at 0.25 \( \mu \text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \) and increased (by 0.25 \( \mu \text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \) each minute) until a fall in BP of at least 20 mmHg was observed.

Data analysis

The analogue outputs from the Finapres and ECG were routed to a dedicated personal computer fitted with a 12-bit analogue–digital converter sampling at 200 Hz per channel. Purpose-written software permitted the recording, calibration and editing of the digitized signal and the derivation for later off-line analysis of beat-to-beat data for systolic, mean arterial and diastolic BP together with the PI from both the Finapres and ECG signals. Temporal resolution was enhanced by a parabolic interpolation between sampling points on the digitized series of both PI signals. PI values were derived primarily from the Finapres signal, with a visual correction facility to allow comparison with the ECG signal. Baseline recordings were scanned by computer for spontaneous sequences of three or more beats in which either BP and PI rose in parallel (+BP/+PI or ‘up’ sequences) or fell in parallel (−BP/−PI or ‘down’ sequences) [4, 6, 19]. We selected sequences on the basis of a stimulus of a minimum change in systolic BP of 0.5 mmHg per beat, but made no similar stipulation regarding the response in order not to bias the analysis towards higher values of BRS. Systolic BP change was correlated with PI change for the immediately succeeding beat (a ‘lag’ of 1) [20, 21] and only sequences with a statistically significant correlation (\( P<0.05 \)) were included in the analysis. The automated analysis derived separate values of BRS for +BP/+PI sequences (up-BRS), −BP/−PI sequences (down-BRS) and pooled results (sequence-BRS). In addition, data were obtained on the average length and correlation coefficient of sequences, the percentage of the entire recording represented by baroreflex activity and the number of sequences of each type.

Power spectral analysis was performed on the baseline recordings after low-pass filtering with an eighth-order Butterworth digital filter with a cut-off frequency of 20 Hz. Recordings with more than four ectopic beats were rejected. The beat-to-beat recordings of systolic BP and PI were interpolated with a third order polynomial and then resampled at a rate of 2 samples/s to render signals with a uniform time base. Linear trends were removed and a 20% tapering cosine window was applied at the extremities of both signals. The signals were transformed to the frequency domain with a fast Fourier transform algorithm using 512 samples. Separate power spectra were computed for systolic BP and PI, and the spectral BRS index \( \alpha \) (spectral-BRS) was computed from the mean of the square roots of the ratios of the spectral powers of PI and systolic BP in the LF (0.05–0.15 Hz) and HF (0.20–0.35 Hz) bandwidths [9, 10]. The cross-spectrum was smoothed with a 9-point triangular window yielding spectral and coherence function estimates with 40 degrees of freedom per subject. The mean coherence in the cross-spectrum of PI and systolic BP was also derived from the three data segments in each subject, with an \( \alpha \) priori lower limit for the coherence set at 0.40 [22].

The BRS was derived for the pharmacological techniques from the linear regression of PI on systolic BP for the ‘ramp’ response using the original method of Smyth et al. [1], but including both inspiratory and expiratory beats [23]. Values were obtained for each of the minimum three effective phenylephrine doses and averaged to derive a final value for phenylephrine-BRS for each individual. Similar analysis of the ‘ramp’ portion of the sodium nitroprusside response yielded a value for nitroprusside-BRS, and the mean of these two values (pharm-BRS) represented the BRS estimated by drug-based techniques in each study subject.

Statistical methods

Spectral analysis parameters are expressed both in absolute terms (either mmHg² or ms²) and after removal of the very-low-frequency component (<0.05 Hz) as normalized units (NU) [7]. All numerical values are expressed either as mean±S.E.M., or as median (interquartile range) for non-parametric data, after testing for normality using the Shapiro–Wilks \( W \) test. Between-group comparisons were made using either Student’s unpaired two-tailed \( t \)-test or the Mann–Whitney test, with a value for \( P<0.05 \) regarded as statistically significant. Agreement between methods is expressed both in terms of linear regression analysis with the 95% confidence interval (CI) for the slope (\( \beta \)) to test the hypothesis of numerical equivalence, and with Bland–Altman plots of the difference between two methods as a function of the mean of the two methods [24].

RESULTS

Of the 22 subjects studied, results from two (one hypertensive, one normotensive) were excluded from analysis because of persistent ectopic activity throughout the study protocol. Table 1 shows the baseline characteristics and clinic BP for the hypertensive and normotensive groups. Clinic heart rate was not significantly different between the two groups.
between normotensive and hypertensive subjects, but values for αHF and spectral-BRS were significantly higher in the normotensive group (Table 3).

### Agreement between methods

Agreement was tested between methods involving a stimulus in the same direction where such a distinction could be made, i.e. with the sequence and pharmacological BRS parameters. Thus nitroprusside-BRS was compared with down-BRS, and phenylephrine-BRS with up-BRS. Pooled results were also compared (pharm-BRS with sequence-BRS). Results of the comparison of pharmacological and sequence methods are shown in Fig. 2. The upper panels show linear regression, with the equivalent Bland–Altman plots in the lower panels.

### Baroreflex sensitivity tests

Table 1 also shows the results from the pharmacological BRS estimation in the study subjects. There was a statistically significant difference between the normotensive and hypertensive groups in the drug-based parameters phenylephrine-BRS, nitroprusside-BRS and the overall pharm-BRS. Within each group, however, there was no significant difference between the BRS from a pressor or a depressor stimulus.

Figure 1 serves to illustrate the sequence analysis technique. Sequence analysis was performed on records of mean total length 1010 ± 29 beats, and the results are given in Table 2. There was no significant difference between hypertensive and normotensive subjects in the number of baroreflex sequences observed or in the percentage of the entire recording represented by baroreflex slopes, but in the normotensive subjects the down sequences tended to be of slightly longer duration. There were no differences between up-BRS and down-BRS within either group, but all sequence-derived BRS parameters were significantly different between normotensive and hypertensive subjects.

Results of spectral analysis are given in Table 3 for the LF and HF bandwidths and the overall α index spectral-BRS. There was no difference in overall PI variability between the hypertensive and normotensive groups [total PI power 782 (512–2159) ms² and 660 (493–1089) ms² respectively, P > 0.2], but there was a significant increase in overall systolic BP variability in the hypertensive group as shown by an increase in total systolic BP power compared with the normotensive group [46.3 (34.6–57.4) mmHg² versus 19.9 (15.8–29.1) mmHg², P = 0.006]. This increased variability was dispersed among all frequency regions, as suggested by the higher absolute but similar relative powers (as NU) for systolic BP within either specified LF or HF frequency region. There was a tendency for values of αHF to be higher than αLF by a mean 2.71 ms/mmHg (P = 0.07). αLF was not different

### Table 1. Baseline characteristics, clinic BP and pharmacological BRS for the hypertensive and normotensive groups. *Different by study design. For definitions of the different parameters of BRS see text. NS, not significant.

<table>
<thead>
<tr>
<th>Hypertensive subjects</th>
<th>Normotensive subjects</th>
<th>P</th>
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<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Age (years)</td>
<td>69 ± 2</td>
<td>68 ± 2</td>
</tr>
<tr>
<td>Clinic</td>
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<td></td>
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<tr>
<td>Systolic BP (mmHg)</td>
<td>160 ± 4</td>
<td>136 ± 3</td>
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<tr>
<td>Diastolic BP (mmHg)</td>
<td>88 ± 2</td>
<td>73 ± 2</td>
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<tr>
<td>Heart rate (beats/min)</td>
<td>65 ± 2</td>
<td>66 ± 3</td>
</tr>
<tr>
<td>Nitroprusside-BRS (ms/mmHg)</td>
<td>3.7 ± 0.7</td>
<td>5.7 ± 0.4</td>
</tr>
<tr>
<td>Phenylephrine-BRS (ms/mmHg)</td>
<td>3.9 ± 0.5</td>
<td>8.6 ± 1.4</td>
</tr>
<tr>
<td>Pharm-BRS (ms/mmHg)</td>
<td>3.8 ± 0.5</td>
<td>7.2 ± 0.9</td>
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Fig. 1. Beat-to-beat recordings of systolic blood pressure (top panel) and pulse interval (PI, middle panel) during one 5 min period. Sequences detected (either +BP/+PI or −BP −PI) are shown as bold lines in the upper panels and as data points with regression lines for individual sequences in the lower panel. Also shown is the mean slope for all sequences (sequence-BRS).
New baroreflex techniques in the elderly

Down-BRS showed significant systematic bias compared with nitroprusside-BRS (mean 3.2 ± 0.7 ms/mmHg higher, \( P = 0.002 \)) but the two values were linearly related, and the slope was not significantly different from unity but with wide confidence limits (regression equation: down-BRS = 2.19 + 1.22 nitroprusside-BRS, \( R^2 = 0.43, P = 0.002; 95\% \) CI for \( \beta = 0.52 - 1.92 \); Fig. 2, left panels). In contrast, up-BRS showed less bias compared with phenylephrine-BRS (mean difference 0.97 ± 0.54 ms/mmHg, \( P = 0.09 \)) but although the two measurements were linearly related, the slope was significantly different from unity, thus rejecting the hypothesis of numerical

| Table 2. Results from sequence analysis for the hypertensive and normotensive groups. %BRS: percentage of the entire recording represented by baroreflex sequences. For definitions of the different parameters of BRS see text. NS, not significant. |
|---------------------------------|-----------------|-----------------|-----------------|
| Hyperensive subjects | Normotensive subjects | \( P \) |
| Down (-BP/-PI) sequences | | | |
| No. of sequences | 41 ± 6 | 39 ± 6 | NS |
| Sequence length (beats) | 5.0 ± 0.2 | 6.4 ± 0.4 | 0.04 |
| Correlation coefficient | 0.95 ± 0.01 | 0.92 ± 0.01 | NS |
| Down-BRS (ms/mmHg) | 5.7 ± 0.7 | 10.1 ± 1.4 | 0.013 |
| Up (+BP/+PI) sequences | | | |
| No. of sequences | 33 ± 6 | 46 ± 6 | NS |
| Sequence length (beats) | 5.4 ± 0.2 | 5.8 ± 0.3 | NS |
| Correlation coefficient | 0.94 ± 0.01 | 0.93 ± 0.01 | NS |
| Up-BRS (ms/mmHg) | 5.0 ± 0.6 | 9.5 ± 1.2 | 0.005 |
| %BRS | 24.4 ± 2.7 | 26.6 ± 4.0 | NS |
| Sequence-BRS | 5.3 ± 0.6 | 9.8 ± 1.2 | 0.006 |

| Table 3. Results from power spectral analysis for the hypertensive and normotensive groups. Data are presented as means ± S.E.M. or median (interquartile range). For definitions of the different parameters of BRS see text. NS, not significant. |
|---------------------------------|-----------------|-----------------|-----------------|
| LF (0.05–0.15 Hz) | | | |
| Systolic BP power (mmHg\(^2\)) | 7.2 (5.6–10.2) | 4.0 (2.7–5.5) | 0.014 |
| PI power (ms\(^2\)) | 29.8 (25.2–34.3) | 35.7 (24.6–45.0) | NS |
| Coherence | 0.52 (0.45–0.65) | 0.53 (0.48–0.54) | NS |
| xLF (ms/mmHg) | 5.0 ± 0.6 | 6.4 ± 0.7 | NS |
| HF (0.20–0.35 Hz) | | | |
| Systolic BP power (mmHg\(^2\)) | 2.3 (1.3–3.5) | 1.0 (0.3–2.8) | NS |
| PI power (ms\(^2\)) | 7.53 (6.58–9.70) | 4.72 (1.72–10.7) | NS |
| Coherence | 0.47 (0.43–0.64) | 0.53 (0.47–0.56) | NS |
| xHF (ms/mmHg) | 4.8 ± 0.5 | 12.1 ± 2.0 | 0.006 |
| Spectral-BRS (mmHg\(^2\)) | 4.9 ± 0.5 | 9.4 ± 1.3 | 0.008 |

Fig. 2. Regression plots (upper panels) and Bland–Altman plots (lower panels) for the comparison of BRS from the pharmacological and sequence methods. Leftmost plots: sodium nitroprusside (SNP) versus down (-BP/-PI) sequences; middle plots: phenylephrine (PE) versus up (+BP/+PI) sequences; rightmost plots: pooled pharmacological (pharm) versus sequence (seq) data. Closed symbols, hypertensive subjects; open symbols, normotensive subjects. Upper panels: dotted line, line of unity (perfect agreement); dashed line, regression line for the whole group. Lower panels: dotted line, mean difference; dashed lines, ± 2 S.D.s of the difference.
equivalence between the results from the two methods \( (\beta = 0.71, \text{95\% CI 0.46-0.97; Fig. 2, middle panels}) \). Comparing the pooled sequence and pharmacological results, there was a significant bias in favour of higher values for sequence-BRS \( (\text{mean difference } 2.1 \pm 0.5 \text{ ms/mmHg, } P = 0.0001) \), but the two variables were linearly related with a slope of close to unity \( (\beta = 1.17, \text{95\% CI 0.85-1.49; Fig. 2, right panels}) \). Within this there were still some results with a large discrepancy; for example one subject with pharm-BRS of 1.8 ms/mmHg and sequence-BRS of 6.1 ms/mmHg. Nonetheless, as shown in Table 2, sequence analysis was consistently able to demonstrate a difference between the hypertensive and normotensive groups in the same manner as the drug-based method.

In view of the overall estimate of BRS represented by the spectral-BRS and the inherent inability of spectral methods to differentiate between rising and falling baroreflex sequences, comparisons were confined to those between spectral-BRS and the pooled results pharm-BRS and sequence-BRS. These are shown in Fig. 3, again depicting linear regression in the upper panels and Bland–Altman plots in the lower. The level of agreement between the different methods was generally very high. Spectral-BRS did show significant positive bias compared with pharm-BRS \( (\text{mean difference } 1.65 \pm 0.46 \text{ ms/mmHg, } P = 0.002) \) and the 95% CI for the regression slope included unity \( (\beta = 1.16, \text{95\% CI 0.80-1.52}) \). Again, there were a few individuals in whom agreement was not close; for example one subject with a pharm-BRS of 8.8 ms/mmHg and spectral-BRS of 17.0 ms/mmHg. Comparison of sequence-BRS with spectral-BRS showed an even closer linear relation between the two parameters \( (\beta = 0.97, \text{95\% CI 0.80-1.14}) \), with no systematic bias (mean difference \( = 0.42 \pm 0.28 \text{ ms/mmHg, } P = 0.15 \)), indicating a high level of agreement between the sequence and spectral parameters and sustaining the hypothesis of numerical equivalence between the methods.

**DISCUSSION**

This study in elderly subjects across a broad range of clinic BPs suggests that results obtained from computerized sequence and power spectral analyses show an acceptable level of agreement with BRS.
obtained from the more traditional pharmacological methods and analysis, with persisting systematic bias in some cases. While the phenylephrine technique was the first of the methods to be described [1], it cannot be regarded as a true ‘reference’ value, and so the issue of bias in one or other direction is confined to whether results obtained by different methods can be regarded as numerically equivalent or interchangeable [24].

The findings from the present study thus agree substantially with those of other workers who have similarly addressed the issue of comparability albeit in groups of younger normotensive or mild hypertensive subjects [6, 8, 9, 11–13]. However, a further examination of these studies requires us to first consider the methodology of comparisons between methods [24]. This has largely been performed using only standard correlation coefficients, a method that solely tests for a linear relationship between two variables (and compares the slope of the relation with the null hypothesis of a slope of zero) and does not assess agreement. We have therefore elected to compare methods using two statistical tests, firstly by comparison of linear regression with a model with a slope of unity (to test for a constant linear relation between methods, i.e. numerical equivalence or complete ‘agreement’), and secondly by examining Bland–Altman plots. The difference between this approach and simple correlation is well illustrated if one considers the available data in the literature. In one recent paper [13] good agreement is claimed between BRS as assessed by the two newer techniques and phenylephrine-BRS on the basis of a statistically significant correlation. An examination of the data, however, demonstrates that the agreement between the two methods is far from good, as seen both from the substantial scatter when the individual data points are plotted with regression slopes significantly different from 1, and from the substantial systematic bias when the methods are compared. In the recent work of Parlow et al. [11] it is possible to assess agreement between the sequence approach and pharmacological BRS measures, and this is shown to be good in a small group of young normotensive subjects, some of whom had extremely high BRS. However, in the one published study which included a comparison of sequence and spectral techniques (using zLF) in a group of eight elderly, mild hypertensive subjects [14] (who tend to show much lower values for BRS), the results are described only as ‘superimposable’ (indicating only that the group means were similar) with no further data given with which to assess agreement. Agreement between results obtained by different methods may also be affected by the reproducibility of the different techniques, a topic also not usually addressed in such studies. In our laboratory the non-invasive methods have a between-visit reproducibility of approximately 17% ($n = 56$), (S. L. Dawson, M. A. James, P. Weston, R. B. Panerai and J. F. Potter, unpublished work), similar to that recently reported in young normotensive subjects [25]. Despite these reservations regarding previous studies, the data from the present study lead us to suggest that the acceptable agreement between the traditional drug-based and newer methods should permit their interchangeability in various clinical and experimental situations, although it does not allow the absolute BRS values obtained in studies using the different techniques to be regarded as equivalent.

Nonetheless it remains that the traditional and newer techniques have important methodological and clinical differences, not the least of which being that the sequence and spectral techniques involve the automated computerized analysis of BP and PI whereas there still remains some potential subjectivity when selecting the segments of interest in the response to pharmacological stimuli despite the use of standardized criteria [1, 23]. In physiological terms the pharmacological method involves the slow onset of an artificial perturbation of the baroreflex beyond the usual limits operating at rest, whereas sequence analysis studies the smaller fluctuations that occur in the subject at rest and free from external stimulation. The majority of sequences seen in the present study were of six or fewer beats, whereas it is the express intent of drug-based methods to provoke a sustained ‘ramp’ of perhaps 15 to 20 beats duration, raising the possibility that the reflex response under these stimulated conditions is different from the much smaller baroreflex-mediated adjustments that occur under basal conditions. Indeed it is this important property of the drug-based method which enables the behaviour of the arterial baroreflex over the full range to be studied, permitting sigmoidal fitting of the stimulus–response relation and allowing parameters such as threshold and saturation ranges to be described [11, 26, 27]. This is inherently not possible with analysis of spontaneous sequences which operate within a relatively narrow pressure range over the linear portion of the baroreflex curve. These points would tend to suggest a potential dichotomy in the approach to BRS estimation. The drug-based techniques, and particularly the phenylephrine injection method, have come to be regarded as the ‘gold standard’ in the assessment of arterial BRS, but this is more likely to be due to their historical precedence than any view that they better describe the spontaneous behaviour of baroreflex mechanisms in the resting subject. Perhaps now it should be accepted that the ‘old’ and ‘new’ techniques have different and complementary roles in extending our understanding of cardiovascular neural control.

Some consideration is required at this point of the different methodologies involved in computerized sequence analysis [4, 6, 19, 21]. Unlike other studies, we have attempted to make the minimum number of stipulations regarding what qualifies as a ‘sequence’, and in particular specified a sequence only in terms of the stimulus (in our case
0.5 mmHg/beat sustained over a minimum of three beats) and not also in terms of the response. To also create minimum criteria for the response (usually a change in PI of 4 ms [4, 19]) would bias the analysis towards higher BRS, particularly with the shorter sequences. Furthermore, in the current study we have stipulated only that the sequence satisfies the criterion of linear dependence between stimulus and response (i.e. a statistically significant regression) and not specified a preset minimum coefficient of determination. Again this protects against the exclusion of sequences of low BRS, a factor which is particularly pertinent in the study of elderly subjects who demonstrate much lower BRS than younger subjects.

The premise upon which spectral analysis of BRS is based is different from that which underpins the other techniques [9, 28–30]. Whereas the time domain methods are based upon a simplified, open-loop model of the transfer function between systolic BP and PI which usually ignores other influences such as respiration and rate of change of pressure, spectral analysis examines the non-causal linkage between the spectra of systolic BP and PI within an integrated closed-loop model of the cardiovascular system which accommodates other potential neural and non-neural influences on both BP and PI [9, 28, 29, 31]. Recent work from our group [32] studying spectral analysis of a linearized model reconstructed from the sequence analysis has shed light on some common features that may account for the agreement between the two techniques, with the LF band being associated with the longer sequences and the HF band corresponding with the shorter sequences. It would therefore seem appropriate when making comparisons between sequence and spectral methods to use the overall spectral index z [9, 10, 32]. We have also recently observed in a group of elderly subjects [33] that after phenylephrine injection, spectral analysis detected substantial increases in power, phase and coherence in the very-low-frequency region corresponding with a slow (approximately 0.025 Hz) oscillation provoked by vasoactive drug injection, and that as a result there was good agreement between phenylephrine-BRS and the spectral ratio z in the very-low-frequency region. This suggests that phenylephrine injection induces a baroreflex-mediated very-low-frequency link between systolic BP and PI that is not present at rest and not described by sequence or spectral analysis of resting recordings, thus accounting in part for the discrepancies between results obtained by different methods.

Study limitations

In our comparison of the different BRS techniques, the potential limitations of the study need to be considered. We chose to make the pharmacological comparison using phenylephrine bolus injection and sodium nitroprusside infusion given that these were the most commonly used techniques on which our understanding of BRS in the elderly was based [16–18, 23], but this does not allow us to directly compare the pharmacological BRS response to a rising and falling systolic BP. In this age group, technical difficulties can arise in data analysis because of low values for BRS and a relatively lower signal-to-noise ratio than in younger subjects, but this study has demonstrated the feasibility as well as the comparability of non-invasive baroreflex studies in the elderly. The use of the Finapres method of BP registration in the elderly has previously been found to be acceptable in comparisons with intra-arterial recordings [34, 35]. Further mention is also required here on the issue of coherence. Most investigators have used a value of 0.50 as an arbitrary threshold for assuming significant linear dependence in the cross-spectrum between systolic BP and PI based on earlier work [28, 36]. This issue is often overlooked in other studies using spectral techniques despite the observation that the sample distribution of coherence is dependent on the number of degrees of freedom available for its estimation at each frequency [37]. We have observed that, for the conditions of our study, the lower 95% CI for coherence is actually 0.40 [22]. However, under other circumstances with fewer degrees of freedom this value may rise to 0.80, and in each case the confidence limits for coherence should be computed and matched to the data analysis procedure.

In conclusion, our study comparing the newer and longer-established techniques in a group of elderly subjects across a broad range of clinic BPs indicates that an acceptable measure of agreement exists between the different methods for the assessment of BRS. This should enable the application of spectral and sequence analyses in clinical and experimental situations where the use of drug injections to provoke substantial BP fluctuations would be inadvisable, for example, in subjects with very high BP, or in conditions such as pre-eclampsia, acute stroke or myocardial infarction. Indeed one could go further and propose that the newer techniques could supersede drug-based methods for the description of the spontaneous behaviour of baroreflex–heart rate mechanisms under basal conditions. However, the pharmacological methods will retain a role where a fuller description of the entire baroreflex stimulus–response relationship is required.

REFERENCES