Comparison between reproducibility and sensitivity of muscle sympathetic nerve traffic and plasma noradrenaline in man

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1. Although plasma noradrenaline and muscle sympathetic nerve traffic have been shown to be suitable markers of sympathetic activity in man, no study has systematically compared the reproducibility and sensitivity of these two indices of adrenergic tone.

2. Reproducibility data were collected in 10 subjects, in whom plasma noradrenaline was assessed by HPLC on blood samples withdrawn from an antecubital vein and efferent postganglionic muscle sympathetic nerve activity was measured by microneurography from a peroneal nerve, together with arterial blood pressure (Finapres technique). Measurements were obtained in a first session (session 1), 60 min later (session 2) and after 14 days (session 3). While muscle sympathetic nerve activity values recorded in the three different experimental sessions were closely and significantly correlated with each other (r always >0.90, P<0.001), noradrenaline showed a less significant correlation between sessions 1 and 2 (r=0.71, P<0.05) or no correlation between sessions 1 and 3 (r=0.45, P not significant).

3. Sensitivity data were collected by evaluating muscle sympathetic nerve activity and noradrenaline values in three different age groups (young, middle-age and old subjects, n=18), in three groups with different blood pressures (normotensive, mild and severe hypertensive subjects, n=30) and in a group of eight subjects before and after a physical training programme, i.e. conditions known to modify sympathetic cardiovascular drive muscle sympathetic nerve activity also appears to change more clearly than noradrenaline.

INTRODUCTION

Among the various methods available to measure sympathetic tone in humans [1, 2] plasma noradrenaline assessment and microneurographic recording of muscle sympathetic nerve traffic in a peroneal or brachial nerve have a special interest. This is because plasma noradrenaline concentration can be provided by most laboratories and easily obtained in the clinical setting. Microneurographic recording of sympathetic nerve traffic, on the other hand, although more difficult to obtain because it requires a greater expertise, is at present the only method allowing sympathetic activity to be directly quantified.

Several studies have examined the reproducibility of the plasma noradrenaline and sympathetic nerve traffic measurements, as well as the ability of these two approaches to detect changes in sympathetic tone [3–9]. No study, however, has systematically compared the reproducibility and sensitivity of these two methods in the same subject. The present investigation was undertaken to address this issue.

METHODS

Reproducibility

This study was performed in 10 subjects (aged 44.9±3.5 years, mean ± SEM) with a mild essential hypertension, i.e. with a clinic diastolic blood pressure repeatedly above 90 mmHg and no evidence of major end-organ damage. In each subject medica-
tion was withdrawn for 4 weeks before the study. Beat-to-beat arterial blood pressure was measured from a finger by a Finapres device [10, 11], heart rate was measured from a standard electrocardiographic lead and efferent postganglionic muscle sympathetic nerve activity (MSNA) was measured by microneurography from a peroneal nerve, according to a procedure described in detail in previous studies [12–14]. Plasma noradrenaline (NA) was evaluated by HPLC (see below) from a blood sample taken from an antecubital vein. All measurements were performed in the morning with the subject supine, after an overnight abstinence from smoking and caffeine intake, a light breakfast and an interval of 40 min from patient instrumentation (session 1). MSNA was recorded along with blood pressure and heart rate for 30 min. The blood sample for NA measurement was withdrawn at the end of the nerve traffic and haemodynamic recording period. The procedure was repeated in the same experimental session, after a 60 min interval (session 2), using the same intravenous cannula employed for session 1. Data were collected in the same fashion in a third experimental session performed 2 weeks later in the absence of treatment (session 3). This allowed us to test the short- and medium-term reproducibility of the measurements. Care was taken to avoid environmental disturbances during each session and to standardize conditions in which measurements were obtained in the different sessions.

### Sensitivity

This study involved three conditions known to modify sympathetic cardiovascular influences: (i) physical training in which sympathetic activity is reduced [15], (ii) aging in which sympathetic activity is increased [2, 16] and (iii) essential hypertension in which sympathetic activity is also increased [1, 2]. The physical training protocol involved eight young sedentary normotensive subjects (aged 17.2±0.9 years) in whom a cannula was placed in an antecubital vein and a microelectrode in a peroneal nerve to measure NA and MSNA respectively, as described above. Blood pressure was again measured by a Finapres device and heart rate by a standard electrocardiographic lead. Measurements were performed in the morning after an overnight abstinence from smoking and caffeine intake, a light breakfast and a 40 min supine rest. The study was repeated after 10 weeks of an endurance training programme (long-distance running for 2 h per day, 5 days per week) which increased maximum work capacity and O2 consumption from 197±15 W to 251±13 W and from 34.1±2.3 ml min⁻¹ kg⁻¹ to 40.8±2.0 ml min⁻¹ kg⁻¹ respectively (P<0.02 for both), and comparisons were made within subjects.

The aging protocol involved 18 normotensive healthy subjects, six aged 16.7±0.7 years, six aged 46.0±1.2 years and six aged 68.0±3.5 years. Blood pressure, heart rate, NA and MSNA were measured as described for the physical training protocol. The study was performed only once and comparisons were made between groups. The hypertension protocol involved 30 male subjects, 10 with a normal blood pressure (aged 48.8±4.0 years), 10 with a mild essential hypertension (aged 50.6±3.0 years) and 10 with a more severe essential hypertension (aged 52.1±2.9 years). In hypertensive subjects medication was withdrawn 2 weeks before the study. No major disease was present besides hypertension and no subject showed evidence of major end-organ damage. Blood pressure, heart rate, NA and MSNA were measured as described for the physical training protocol. As for the aging protocol the study was performed only once and the comparisons were made between groups.

### Data analysis

In each subject systolic blood pressure, diastolic blood pressure and heart rate values were all averaged over the recording period. Mean arterial pressure was derived from the diastolic blood pressure plus one third of the differential blood pressure value. MSNA over the monitored period was expressed as number of bursts corrected for the concomitant heart rate values (bursts/100 heartbeats). Quantification of MSNA by this approach was shown to be highly reproducible, i.e. to differ by only 3.8% when assessed on the same tracing on two separate occasions by a single investigator [17]. Data were calculated by a single investigator unaware of the experimental design. NA was measured by HPLC [18]. The method enabled the detection of NA changes of 5 pg/ml and the mean coefficient of variation of values obtained from the same sample was 5.4%. These data for sensitivity and reproducibility are similar to those reported by others [5, 7, 18].

Data obtained in individual subjects were averaged and expressed as means±SEM. In the reproducibility study the association between values obtained in the three different experimental sessions was tested by means of Pearson's correlation coefficient. In the reproducibility and sensitivity studies data obtained in different groups were compared by two-way analysis of variance, using Student's t-test with Bonferroni's correction to locate the statistical significance of the differences. A P<0.05 was taken as the level of statistical significance. The study protocol was approved by the ethics committee of the institutions involved and all subjects agreed to participate in the study after being informed about its nature and purpose.
RESULTS

Reproducibility

As shown in Fig. 1, average values of mean arterial pressure, heart rate and MSNA were superimposable in the first recording session (session 1), the recording session run 60 min later (session 2) and the recording session run 14 days later (session 3). Average NA values were slightly less in the recording session performed 60 min after the first one (session 2) and showed a further small reduction 14 days later (session 3). The differences between the average values obtained in the three sessions, however, were not statistically significant.

The correlation between individual MSNA values was extremely high between sessions 1 and 2 and somewhat lower but still high between sessions 1 and 3. In both instances the correlations were much higher than the corresponding correlations between NA values, which were not significant between sessions 1 and 3 (Fig. 2). Correlations between mean arterial pressure values observed in the three sessions were also high and similar to the MSNA correlations (Fig. 3). This was also the case for heart rate values (Fig. 3). In session 1, MSNA and NA showed a significant although weak correlation \(r = 0.62, P < 0.05\). The correlation was not significant in sessions 2 and 3.

Sensitivity

Table 1 shows the blood pressure, heart rate, MSNA and NA values at different ages, at different blood pressures and before and after physical training. In young, middle-age and elderly normotensive subjects blood pressure and heart rate were similar, NA was slightly higher only in the elderly subjects, while MSNA showed a progressive and significant increase from the youngest to the oldest group. In age-matched normotensive, mild and severe hypertensive subjects the progressive increase in mean arterial pressure was accompanied by no difference in heart rate and by a progressive increase in MSNA, while NA was significantly greater only in the severe hypertensive group as compared with the normotensive group. In sedentary subjects physical training caused a slight and significant reduction in mean arterial pressure, a slight non-significant reduction in heart rate and a
marked fall in MSNA, while NA was not significantly reduced as compared with the pretraining condition.

**DISCUSSION**

In the subjects of our study MSNA correlated to an extremely close degree when measured in two different sessions spaced by a 60 min interval (i.e. sessions 1 and 2). Although less close, the correlation was still high when the initial MSNA values were compared with the values obtained 14 days later (i.e. sessions 1 and 3). In contrast, the correlation between NA values obtained in the two sessions spaced by a 60 min time interval (sessions 1 and 2) was barely significant, and the NA values obtained before and after 14 days (i.e. sessions 1 and 3) showed marked differences and no statistically significant relationship. This allows us to conclude that MSNA assessment of sympathetic tone has a better short- and medium-term reproducibility than NA assessment.

Previous data by our group and others have shown that physical training is accompanied by a reduction in sympathetic activity [15, 19] while aging and severity of hypertension are associated with progressive increases in sympathetic activity [2, 20, 21]. In our study this was more consistently reflected by MSNA than by NA which either did not show any significant change (physical training) or changed significantly only when the differences between groups were pronounced, i.e. young versus old or normotensive versus severe hypertensive subjects. Thus, MSNA provides not only greater reproducibility but also a clearer and more consistent reflection of changes in sympathetic activity caused by changes in subjects' status or by disease. This occurs

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**Table 1. Haemodynamic values, MSNA and NA in different subjects or conditions.** Data are shown as means ± SEM. Figures in parentheses refer to number of subjects examined. Statistical significance: *P < 0.05, **P < 0.01.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Mean arterial pressure (mmHg)</th>
<th>Heart rate (beats/min)</th>
<th>MSNA (bursts/100 heartbeats)</th>
<th>NA (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young [6]</td>
<td>95.5 ± 0.6</td>
<td>64.5 ± 4.1</td>
<td>[29.8 ± 5.2]</td>
<td>256 ± 42</td>
</tr>
<tr>
<td>Middle-aged [6]</td>
<td>97.5 ± 2.0</td>
<td>66.8 ± 2.5</td>
<td>[53.9 ± 4.1 **]</td>
<td>193 ± 21</td>
</tr>
<tr>
<td>Old [6]</td>
<td>100.3 ± 6.4</td>
<td>64.3 ± 2.7</td>
<td>[72.8 ± 6.6]</td>
<td>345 ± 54</td>
</tr>
<tr>
<td>Normotension [10]</td>
<td>[97.4 ± 3.0]**</td>
<td>69.9 ± 2.8</td>
<td>[39.4 ± 3.7]</td>
<td>217 ± 18</td>
</tr>
<tr>
<td>Mild hypertension [10]</td>
<td>[90.6 ± 1.9]**</td>
<td>71.0 ± 2.2</td>
<td>[56.0 ± 4.2 **]</td>
<td>230 ± 22</td>
</tr>
<tr>
<td>Severe hypertension [10]</td>
<td>[18.8 ± 2.8]</td>
<td>69.4 ± 1.8</td>
<td>[70.0 ± 5.5]</td>
<td>294 ± 40</td>
</tr>
<tr>
<td>Before training [8]</td>
<td>[97.4 ± 2.3</td>
<td>62.0 ± 3.4</td>
<td>[32.0 ± 2.9 **]</td>
<td>264 ± 35</td>
</tr>
<tr>
<td>After training [6]</td>
<td>97.1 ± 2.8</td>
<td>58.7 ± 3.4</td>
<td>22.0 ± 3.0 **</td>
<td>224 ± 20</td>
</tr>
</tbody>
</table>
Noradrenaline and sympathetic nerve traffic

both when sympathetic activity is increased and when it is reduced.

The reasons why MSNA has a greater reproducibility and more clearly reflects changes in sympathetic drive associated with different conditions and diseases than NA measurements are beyond the goal of the present study. A traditional explanation is that NA spilling into plasma is only a small fraction of the neurotransmitter secreted from sympathetic nerve endings, changes in NA represent only a pale reflection of the neural events [22, 23] which are more reliably assessed by a direct approach such as nerve recording. However, it is possible that MSNA measurements were more reproducible because they were averaged over relatively long time periods and that assessment of NA by averaging values from more than a single blood sample could result in a close reproducibility of this biochemical method as well. This could reduce random alterations and also give a clearer display of changes in sympathetic drive in different conditions and diseases. It should be emphasized, however, that this would require a modification of the procedure used for NA assessment as compared with current standards which are largely based on a single NA measurement.

In conclusion, our data provide evidence that microneurography has a better short- and medium-term reproducibility than NA. By reducing random alterations in the measured values it allows a more consistent detection of changes in sympathetic drive. However, our data should not be interpreted to mean that microneurography is superior and therefore preferable to the NA approach in assessing sympathetic tone. First, although the changes associated with training, aging and hypertension were more consistently reflected by MSNA than by NA, the trend was similar for the two variables which suggests that, whenever the sample is large enough, the two methods might yield similar statistical results. Furthermore, in the clinical setting microneurography is a much less easy and more time-consuming technique to perform than NA assessment. Finally, an obvious limitation of the microneurographic approach is that it only provides information on sympathetic tone to skeletal muscle circulation. Evidence has been obtained that sympathetic drive is similar in different skeletal muscle districts [4, 24], but the relationship between sympathetic tone to skeletal muscle and visceral circulations (e.g. renal and splanchnic areas) remains largely inferential. This should be taken into account when interpreting the MSNA results. It should also prompt the conclusion that NA, a less reproducible and sensitive but more generalized marker of sympathetic drive, is nevertheless still necessary for quantification of sympathetic tone in humans.

REFERENCES