Human muscle sympathetic activity and cardiac catecholamine spillover: no support for augmented sympathetic noradrenaline release by adrenaline co-transmission

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INTRODUCTION

Adrenaline, the principal hormone produced by the adrenal medulla, is also found in low concentrations in extra-adrenal tissues particularly within sympathetic nerves [1, 2]. The functional role of this adrenaline is unclear. There is evidence from animal studies to suggest that adrenaline may stimulate presynaptic β2-adrenergic receptors, increasing the amount of noradrenaline released per nerve impulse [3–6]. These observations underlie the ‘adrenaline hypothesis’ of hypertension pathogenesis [4, 7, 8]. Regional release of adrenaline is evident in humans in several contexts, although not studied to date in essential hypertension. Measurable amounts of adrenaline are released from the heart to plasma in young men during aerobic exercise [9, 10], in elderly men at rest [11], and from the heart [12] and other organs in human heart failure [9, 13].

In several animal species, tissue incubated in a similar concentration of adrenaline to that found in human plasma demonstrates uptake of adrenaline into the sympathetic nerves and release of this adrenaline with nerve stimulation [3, 4, 14]. Binding of released adrenaline to the presynaptic β2-adrenoceptor has been observed to facilitate the stimulation-induced efflux of noradrenaline in animals, with adrenaline thus acting as a co-transmitter. This is of interest because it lends further support to the hypothesis that adrenomedullary activation might contribute to the development of stress-induced hypertension. Likewise, presynaptic β2-adrenoreceptors might be a site of action of β2-adrenergic antagonists in the treatment of hypertension.

The data on whether adrenaline may be a sympathetic neuronal co-transmitter in humans are extensive but conflicting [15–21]. Although previous reports have suggested that sympathetic effector responses to different stressors may be augmented during an adrenaline infusion, this provides no direct evidence that adrenaline acts as a
co-transmitter. In the present study we measured the changes in haemodynamics, cardiac and total body noradrenaline and adrenaline spillovers to plasma and muscle sympathetic activity during a period of isometric exercise before and after an adrenaline infusion. Our aim was to determine whether after loading sympathetic nerves with adrenaline there is facilitation of the release of noradrenaline at rest and in response to a stressor.

**METHODS**

**Material**

We studied eight healthy male volunteers recruited from the general community by advertisement. They had a mean age of 21 (range 19–23) years and body mass index of 25 ± 1 (21–30). A comprehensive clinical and laboratory evaluation was performed on each subject to establish that they were in good health. All subjects gave their written informed consent and the study was approved by the Alfred Hospital Ethics Review committee.

**Procedure**

Total body and cardiac noradrenaline spillovers were assessed by radiotracer kinetic techniques and microneurography was used to measure muscle sympathetic activity. The study design is summarized in Fig. 1. Subjects were studied supine in the mornings after a standardized light breakfast, alcohol and caffeine having been excluded for the previous 12 h. The nerve recording started approximately 3 h after the meal. For plasma catecholamine sampling a brachial or radial artery cannula and a central venous catheter were inserted percutaneously under local anaesthesia. The venous catheter, a Webster coronary sinus flow catheter (Webster Laboratories Inc, CA, U.S.A.), was placed under fluoroscopic control in the coronary sinus. Radiolabelled adrenaline (1-N-methyl-3H) and noradrenaline (1-7-3H) (New England Nuclear, Boston, U.S.A.) were infused at a rate of 0.5–1.0 μCi/min into a forearm vein, for a minimum of 60 min before blood sampling (Fig. 1), to ensure that steady-state plasma concentrations had been reached [22]. Arterial and coronary sinus plasma samples (5 ml) for catecholamine assays were obtained at rest, during the last 2 min of the isometric handgrip test, during the last 2 min of the 1 μg adrenaline infusion, during the last 2 min of the highest dose of the adrenaline infusion given, at rest approximately 30 min post-infusion, and during the last 2 min of the second isometric test (Fig. 1). Samples were transferred immediately to ice-chilled tubes containing EGTA and reduced glutathione, centrifuged at 4°C, and stored at −70°C before assay.

**Sympathetic nerve recording**

Multi-unit post-ganglionic sympathetic activity was recorded with a tungsten micro-electrode (Titronics Medical Instruments, Iowa City, Iowa, U.S.A), with an uninsulated tip diameter of approximately 1 μ inserted in a muscle fascicle of the peroneal nerve at the fibular head. A reference electrode was inserted subcutaneously a few centimetres away from the recording electrode. The neurogram was amplified (× 50 000), filtered (bandpass 500–2 kHz), passed through a discriminator for further noise reduction, and audiomonitored. A mean voltage display was

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**Fig. 1. Diagrammatic presentation of the study design: R, rest; ISOM, isometric testing; WASHOUT, 20 min washout period after adrenaline infusion. Adrenaline infusion was given in incremental doses depending on tolerance. Starting dose 1 μg/min for 10 min (n = 8), 2 μg/min for at least 10 min (n = 8) and progressing to 3 μg/min if tolerated for at least 25 min (n = 6); where 3 μg dose was not tolerated these subjects continued on the 2 μg dose for a total of 25 min. Infusion scale is interrupted to indicate a variable time course.**
been described previously [23]. The mean voltage brush 280; Gould Recording Systems Division, other variables on an 8-channel ink recorder (Gould Cleveland, OH, U.S.A.) throughout the experiment. For analysis, sympathetic bursts were identified visually in the mean voltage neurogram and expressed as bursts/min. The values presented are the mean of 2 min taken from the same time periods as the plasma samples were obtained. In addition, each minute was analysed during the period after ceasing the adrenaline infusion.

Catecholamine kinetics

The rates of noradrenaline and adrenaline spillover from the heart were calculated according to the Fick principle, corrected for the fractional extraction of 3H-labelled catecholamine across the heart:

\[
\text{Cardiac CAT spillover} = [(\text{CAT}_{\text{a}} - \text{CAT}_{\text{s}}) + \text{CAT}_{\text{a}} \times \text{CAT}_{\text{ex}}] \times \text{CSPF}
\]

where CAT is catecholamine, CAT_{sa} is plasma catecholamine concentration in the coronary sinus, CAT_{a} is arterial plasma catecholamine concentration, CAT_{ex} is the steady-state fractional extraction of plasma tritiated catecholamine across the heart, and CSPF is the coronary sinus plasma flow (ml/min). Coronary sinus plasma flows were derived from thermodilution-determined blood flows and the haematocrit.

The fractional extraction of tritiated catecholamine from plasma at steady state during passage through the heart was calculated from the equation

\[
\text{CAT extraction} = \frac{[3H]\text{CAT}_{\text{a}} - [3H]\text{CAT}_{\text{ex}}}{[3H]\text{CAT}_{\text{a}}}
\]

Adrenaline infusion

The adrenaline infusion dose was titrated to the subject's response. Each subject commenced with at least 10 min infusion at a rate of 1 µg/min. The infusion was increased to 2 µg/min for at least 10 min and if this was tolerated (i.e. the increase in pulse rate was no more than 20 beats/min and the subject was asymptomatic) then the rate was increased to 3 µg/min for at least 25 min. Six subjects received 3 µg/min which was the maximum infusion rate given. If 3 µg/min was not tolerated (n = 2), then the subject continued on 2 µg/min for a total of at least 25 min. The whole duration of adrenaline infusion was approximately 45 min and the total infused quantity approximately 1355 ng/kg.

Isometric handgrip

The isometric handgrip contraction was performed for 10 min at 30% of the subjects' predetermined maximum capacity. This was a sustained contraction which required constant encouragement and support from the investigator in order for the contraction to be maintained. Plasma flows were determined after minute 7 and were completed by minute 9. Plasma samples were obtained and the neurogram was analysed during minutes 9 and 10.

Statistics

Results are expressed as means ± S.E.M. Analysis of variance with Dunnett's multiple-range test was used to compare the changes in mean values over time during the adrenaline infusion and during the washout phase. Student's t-test was used to compare periods pre- and post-adrenaline infusion; P < 0.05 was considered significant.

RESULTS

Changes during adrenaline infusion

Systolic blood pressure rose progressively with increasing doses of adrenaline from 141 ± 6 mmHg at rest to 150 ± 10 mmHg at the 3 µg/min infusion rate (P < 0.01). Diastolic blood pressure decreased from 72 ± 3 mmHg at rest to 68 ± 3 mmHg at 1 µg/min (P < 0.05) and to 64 ± 4 mmHg at 3 µg/min (P < 0.01). Heart rate increased during the infusion from 66 ± 2 beats/min at rest to 72 ± 2 beats/min at 1 µg/min (P < 0.05) and to 79 ± 3 beats/min at 3 µg/min (P < 0.01). The results are summarized in Fig. 2.

Total noradrenaline spillover increased during the infusion, with a maximum change from rest at 3 µg/min (Table 1). Cardiac noradrenaline spillover increased from 13.9 ± 3.9 to 22.4 ± 5.0 pg/ml at 3 µg/min. The extraction rates of both adrenaline and noradrenaline across the heart decreased significantly at 3 µg/min (P < 0.01); this may be related to a 38% increase in coronary sinus blood flow which occurred during the infusion. Arterial noradrenaline concentrations increased from a control value of 223 ± 39 to 301 ± 49 pg/ml at the 3 µg/min infusion rate (P < 0.05). Similarly, the noradrenaline concentration in the coronary sinus increased from 162 ± 32 pg/ml before the infusion to 226 ± 41 pg/ml at 3 µg/min (P < 0.05).

During the adrenaline infusion there was an increase in sympathetic nerve traffic to muscle from
a resting level of 21 ± 3 to 28 ± 6 bursts/min at the 3 μg/min infusion rate (P < 0.05) (Fig. 2).

**Adrenaline washout period**

After the adrenaline infusion was stopped the arterial plasma adrenaline concentration rapidly diminished towards pre-infusion levels, as did blood pressure and heart rate (Fig. 3). There was a marked rise in muscle sympathetic activity during the washout phase with a peak value of 45 ± 1 bursts/min at minute 4 (P < 0.01) followed by a progressive return towards pre-infusion values, which were reached approximately 20 min after the end of the infusion (Fig. 3).

**Cardiac adrenaline uptake**

To assess whether the amount of adrenaline infused could be expected to have an effect at a pre-

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Fig. 2. Sequential changes during the adrenaline infusion expressed as mean data (± S.E.M.) for preceding rest period, 1 μg infusion and pooled data for 2-3 μg infusion. Haemodynamic measurements on the left panel and spillover measurements (NA, noradrenaline) and muscle sympathetic nerve activity (MSA) on the right. *P < 0.05, ▲P < 0.01, indicates significant change from rest. Reflex sympathetic nervous stimulation was evident during the adrenaline infusion.
Adrenaline is not a co-transmitter in humans.

Table 1. Total noradrenaline spillover. Mean total noradrenaline spillover data (± S.E.M.) at rest, during isometric handgrip before the adrenaline infusion, rest pre-adrenaline infusion, adrenaline infusion (1 µg/min and 3 µg/min), rest post-adrenaline infusion and the final isometric period after the adrenaline infusion. *P < 0.009 indicates a significant change from the corresponding rest period. †P < 0.01 indicates significant change from the rest period immediately before the adrenaline infusion. There was no significant difference between rest periods, nor was there a significant difference between isometric values.

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Isometrics</th>
<th>Rest pre-infusion</th>
<th>Adrenaline infusion (1 µg/min)</th>
<th>Adrenaline infusion (3 µg/min)</th>
<th>Rest</th>
<th>Isometrics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total noradrenaline spillover (ng/min)</td>
<td>589 (± 120)</td>
<td>1280 (± 208)*</td>
<td>618 (± 119)</td>
<td>763 (± 105)</td>
<td>940 (± 55)†</td>
<td>698 (± 98)</td>
<td>987 (± 162)</td>
</tr>
</tbody>
</table>

Table 2. Adrenaline uptake/storage in the heart. Data outlining estimated adrenaline uptake/storage in the heart during the adrenaline infusion based on the cold adrenaline extraction (arterial adrenaline concn. – coronary sinus adrenaline concn./arterial adrenaline concn.). Estimated cardiac uptake = cold adrenaline extraction × plasma flow × adrenaline concn. The total µg is the minimum amount based on the minimum infusion time.

<table>
<thead>
<tr>
<th>Infusion dose</th>
<th>1 µg</th>
<th>2–3 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial adrenaline concn. (µg/ml)</td>
<td>368 (24)</td>
<td>726 (102)</td>
</tr>
<tr>
<td>Coronary sinus adrenaline concn. (µg/ml)</td>
<td>141 (17)</td>
<td>331 (57)</td>
</tr>
<tr>
<td>Transcardiac plasma adrenaline fractional extraction</td>
<td>0.617</td>
<td>0.544</td>
</tr>
<tr>
<td>Coronary sinus plasma flow (ml/min)</td>
<td>156 (28)</td>
<td>164 (15)</td>
</tr>
<tr>
<td>Estimated cardiac uptake (µg/min)</td>
<td>0.035</td>
<td>0.065</td>
</tr>
<tr>
<td>Time (min)</td>
<td>18.25</td>
<td>28</td>
</tr>
<tr>
<td>Total adrenaline uptake (µg)</td>
<td>0.65</td>
<td>1.81</td>
</tr>
</tbody>
</table>

Synaptic level we determined the cardiac uptake/storage of adrenaline in the heart (Table 2). We found that the mean total uptake of adrenaline into cardiac tissue for the 1 µg/min infusion rate was 0.65 µg, and for the 2–3 µg/min rate it was 1.81 µg. This is an underestimate of the true amount of adrenaline taken up by the heart because in those subjects who went on to have the 3 µg/min infusion (n = 6) there was no measurement of uptake during the 2 µg/min infusion. Thus, the minimum mean total adrenaline uptake by the heart was 2.46 µg.

Effects of prior adrenaline infusion on values at rest

Twenty minutes after the adrenaline infusion was stopped all haemodynamic parameters had returned to baseline levels (Fig. 3). Systolic blood pressure was 143 ± 4 compared with 144 ± 4 mmHg at the initial rest period before the first isometric test. Diastolic blood pressure was 75 ± 3 compared with 74 ± 3 mmHg, and heart rate was slightly higher post-infusion at 70 ± 3 versus 65 ± 3 beats/min but this was not a significant difference. Muscle sympathetic nerve activity was similar post- and pre-infusion (24 ± 4 compared with 21 ± 3 bursts/min, P not significant). Resting post-infusion cardiac noradrenaline spillover (16.2 ± 2.8 ng/min) was not statistically different from the resting pre-infusion value.
Effects of prior adrenaline infusion on responses to handgrip

The results obtained during isometric handgrip before the adrenaline infusion are consistent with previously published evidence of sympatho-excitation in response to isometric challenge [24, 25]. There were significant increases (P<0.01) in heart rate (from 65±3 to 84±3 beats/min), systolic blood pressure (from 144±4 to 173±6 mmHg) and diastolic blood pressure (from 74±3 to 98±4 mmHg) (Fig. 4). Muscle sympathetic activity increased from 23±3 to 35±2 bursts/min (P<0.01). There was a significant increase in the adrenaline secretion rate, from 220±54 to 740±118 ng/min (P<0.002). Total noradrenaline spillover increased from 589±120 to 1280±208 ng/min (P<0.02) and coronary sinus noradrenaline spillover increased from 13.9±3.9 to 39.6±12.9 ng/min (P<0.01) (Fig. 5).

Isometric handgrip after the adrenaline infusion caused no augmentation of responses (Figs 4 and 5). The blood pressure and heart rate increases were similar to the pre-infusion values, as were the changes in noradrenaline spillover. Muscle sympathetic activity tended to increase (from 24±11 to 36±16 bursts/min, P=0.07). Cardiac noradrenaline spillover increased to a similar degree as pre-adrenaline (from 16.2±2.8 to 39.6±13.0 ng/min, P<0.05), and total noradrenaline spillover tended to increase (from 698±98 to 987±162 ng/min, P=0.07) (Table 1). Although the P value for the increase in total noradrenaline spillover and muscle sympathetic activity was only 0.07, there is the possibility that a type II error exists and this may be corrected by increasing the sample size.

**DISCUSSION**

As outlined earlier there is evidence from isolated animal tissues that adrenaline can be taken up into sympathetic nerves and increase the amount of noradrenaline released per nerve impulse from the terminal. The data in humans, although extensive [15–21], is conflicting. The aim of the present study was to search for functional evidence that in intact humans, adrenaline can be taken up into sympathetic nerves, released as a co-transmitter, and facilitate the release of noradrenaline. To this end we infused adrenaline intravenously to load sympathetic nerves with adrenaline, and measured total body and cardiac noradrenaline spillover during isometric handgrip contraction, a manoeuvre known to increase sympathetic nerve activity. As an index of sympathetic nerve traffic we monitored muscle sympathetic activity in a leg nerve. Our main finding is that no evidence of facilitated noradrenaline release was obtained 30–40 min after the end of the adrenaline infusion, neither at rest nor during isometric handgrip.

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**Table 3. Cardiac noradrenaline kinetics.** Mean noradrenaline spillover data (±S.E.M.) during rest and isometrics, pre- and post-adrenaline infusion. *P<0.05 indicates significant change from corresponding rest period.

<table>
<thead>
<tr>
<th>Plasma noradrenaline concn. (pg/ml)</th>
<th>Transcardiac plasma [3H]noradrenaline extraction</th>
<th>Plasma flow (ml/min)</th>
<th>Cardiac noradrenaline spillover (ng/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial</td>
<td>Coronary sinus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-adrenaline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>223 (±39)</td>
<td>0.79 (±0.04)</td>
<td>118 (±22)</td>
</tr>
<tr>
<td>Isometrics</td>
<td>414 (±44)</td>
<td>0.66 (±0.05)</td>
<td>192 (±35)</td>
</tr>
<tr>
<td>Post-adrenaline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>228 (±31)</td>
<td>0.76 (±0.03)</td>
<td>118 (±20)</td>
</tr>
<tr>
<td>Isometrics</td>
<td>360 (±57)</td>
<td>0.68 (±0.04)</td>
<td>162 (±29)</td>
</tr>
</tbody>
</table>

**Table 4. Cardiac adrenaline kinetics.** Mean adrenaline spillover data (±S.E.M.) during rest and isometrics, pre- and post-adrenaline infusion. *P<0.05 indicates significant change in rest periods, and †P<0.05 indicates a significant change in isometric periods.

<table>
<thead>
<tr>
<th>Plasma adrenaline concn. (pg/ml)</th>
<th>Transcardiac plasma [3H]adrenaline extraction</th>
<th>Plasma flow (ml/min)</th>
<th>Cardiac adrenaline spillover (ng/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial</td>
<td>Coronary sinus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-adrenaline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>103.38 (±21.8)</td>
<td>0.51 (±0.04)</td>
<td>118 (±22)</td>
</tr>
<tr>
<td>Isometrics</td>
<td>250 (±40)</td>
<td>0.37 (±0.05)</td>
<td>192 (±35)</td>
</tr>
<tr>
<td>Post-adrenaline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>89.5 (±11.37)</td>
<td>0.48 (±0.03)</td>
<td>118 (±20)</td>
</tr>
<tr>
<td>Isometrics</td>
<td>160 (±24)</td>
<td>0.39 (±0.03)</td>
<td>162 (±29)</td>
</tr>
</tbody>
</table>
Methodological considerations

Due to the complexity of the study design, and its invasive nature, there were no time control subjects in the experiment, and the sequence of the interventions was fixed. In other respects, the experimental design had several advantages. Previous studies investigating the putative facilitatory effect of adrenaline on noradrenaline release in human sympathetic nerves have monitored plasma noradrenaline concentrations and/or sympathetic effector variables as measures of the effects [15, 16, 18, 20, 21, 26, 27]. Plasma noradrenaline concentrations, however, may provide ambiguous data because they are determined not only by noradrenaline release but also by the clearance of noradrenaline from the plasma. In addition, noradrenaline concentrations in arterial and antecubital venous plasma give no information about effects in individual organs. Sympathetic end-organ effector responses may also be misleading since they can be influenced by non-neural (e.g. circulating, mechanical, metabolic) stimuli. Since we used direct determination of noradrenaline spillover, we avoided the disadvantages of these more indirect approaches.

The heart was chosen for particular study because its sympathetic nerves avidly extract adrenaline from

Fig. 4. Sequential changes during isometric testing in systolic blood pressure, diastolic blood pressure and heart rate before and after the adrenaline infusion. The solid bars represent the mean value during the isometric periods. The shaded bars represent the change from baseline during both isometric periods.

Fig. 5. Sequential changes during isometric testing in cardiac noradrenaline (NA) spillover, muscle sympathetic nerve activity (MSA), and total noradrenaline (NA) spillover before and after the adrenaline infusion. The solid bars represent the mean value obtained during the isometric periods and the shaded bars represent the change from baseline during these two periods. Note - there are no significant changes in the parameters measured between the isometric periods, indicating that the adrenaline infusion did not augment the sympathetic response to isometric challenge.
plasma [28]. This meant that we were unable, however, to record noradrenaline spillover and sympathetic nerve traffic from the same tissue. In the absence of measurements of firing rates of cardiac sympathetic nerves our conclusions rest on the use of muscle sympathetic activity as a surrogate measure of the strength of cardiac sympathetic activity. There is some experimental support for this usage. In a small sample of resting subjects we recently found evidence of a positive correlation between the strengths of the sympathetic neural drives to heart and skeletal muscle [24]. Furthermore, isometric handgrip contractions induced similar fractional increases of both cardiac noradrenaline spillover and sympathetic traffic to leg muscles, even if there is no evidence of a direct proportionality between the increases in individual subjects [24].

Loading of neuronal stores with adrenaline

Mean adrenaline uptake by the heart during the adrenaline infusion was at least 2.46 μg. Prior studies utilizing the pharmacological inhibition of neuronal catecholamine uptake by the tricyclic antidepressant desipramine [28] indicate that approximately 82% of the uptake of adrenaline from plasma into the heart is into sympathetic nerves, while 18% is into extraneuronal tissues. Accordingly, mean uptake of adrenaline into the sympathetic nerves of the heart was at least 2 μg. The adrenaline content of the healthy human heart is somewhat uncertain, but a value of 0.1 μg/g is probable (M. R. Bristow, personal communication). Given this, our adrenaline infusion presumably increased cardiac neuronal adrenaline stores by approximately 12.5%. After the infusion, at a time when the plasma concentration of adrenaline had returned to baseline, adrenaline spillover from the heart was evident at rest. This is in keeping with our loading of cardiac neuronal stores with adrenaline, and the relatively slow metabolism and long half-life of adrenaline in the heart [28].

Method of determining catecholamine kinetics

While isotope dilution methodology to measure noradrenaline release by the heart [29] is well validated and has been used extensively, application of the technique to measure cardiac adrenaline release is a recent innovation. Simultaneous measurement of release of both noradrenaline and adrenaline from the heart is feasible [30], but the technical difficulties are greater for adrenaline. Plasma concentrations of adrenaline are lower, especially in the coronary sinus, and assay sensitivity must be adequate for plasma concentrations of the order of 30–60 pg/ml. This applied in the present study. A theoretical prerequisite for radioisotope determina-

tion of regional catecholamine spillovers is that there is negligible release of the tracer from an organ after its uptake from plasma. This has been demonstrated for noradrenaline [31], where the tissue-specific radioactivity is low. For adrenaline in the heart, where the pool size of the unlabelled catecholamine is much lower than for noradrenaline, there is no proof to-date that this prerequisite is fully met. In fact, the small negative values obtained in the present study for adrenaline spillover from the heart before the adrenaline infusion probably signify both very low intrinsic rates of cardiac adrenaline release, and some degree of recycling of the tracer [32]. Release of tritiated adrenaline from the sympathetic nerves of the heart would lead to underestimation of cardiac adrenaline spillover rates. Despite this potential difficulty, cardiac release of adrenaline has previously been demonstrated with this methodology in several studies (see Introduction).

Effects of the adrenaline infusion

The adrenaline infusion led to similar haemodynamic effects as seen in previous studies [20, 21], with increases of heart rate and systolic blood pressure and a decrease of diastolic blood pressure. We also found increased total noradrenaline spillover and arterial plasma noradrenaline concentration. This was probably due to sympatho-excitation, as evidenced by the increase of muscle sympathetic activity at the 3 μg/min adrenaline infusion rate. Similar findings were reported by Persson et al. [27]. Since the strength of muscle sympathetic activity varies inversely with changes of diastolic blood pressure [33], the reduction of diastolic blood pressure during the adrenaline infusion probably contributed to the increases of nerve traffic, total noradrenaline spillover and plasma noradrenaline concentration.

The termination of the adrenaline infusion was associated with a transient increase of muscle sympathetic activity which returned towards pre-infusion levels after approximately 20 min. A similar but more prolonged increase was seen by Persson et al. [27], who suggested that it was due, in part, to a reduction of central venous pressure. The shorter duration of the sympatho-excitation in our study was probably related to our adrenaline dose being smaller than that used by Persson et al. [27] (last infusion rates 3 versus 5.7 μg/min, total adrenaline doses approximately 1355 versus 8640 ng/kg).

The findings during the pre-adrenaline isometric handgrip contraction confirm our previous observations, that the increases of blood pressure and heart rate evoked by this manoeuvre are associated with simultaneous increases of sympathetic nerve traffic to resting leg muscles and increases of total body and cardiac noradrenaline spillover [24]. The effects were similar during the post-adrenaline handgrip contraction, even if the increases of total noradrena-
line spillover and muscle sympathetic activity did not reach full statistical significance ($P = 0.07$ for both).

Noradrenaline spillover is a function of the number of sympathetic impulses in the sympathetic nerves, the amount of noradrenaline liberated per impulse, and the extent of neuronal reuptake of the transmitter after its release. Around 30 min after the end of the adrenaline infusion noradrenaline spillover (total and cardiac) and noradrenaline plasma concentrations had returned to pre-infusion values. In addition, the handgrip-evoked effects were similar before and after the adrenaline infusion. Assuming that any adrenaline-induced increase of cardiac sympathetic traffic during the infusion also had returned to pre-infusion values (as did muscle sympathetic activity), the present data provide strong evidence for an unchanged noradrenaline release/sympathetic impulse in adrenaline-loaded sympathetic nerves. Since the sympathetic system may be activated in a differentiated manner we cannot exclude the theoretical possibility that the adrenaline infusion led to a reduction of resting activity in the nerves to some sympathetic subdivision, which had not returned to pre-infusion values at the time of our measurements. If so, a selective facilitation of noradrenaline release in those nerves could still be present. The same argument applies if the handgrip-evoked activation was lower in some sympathetic subdivision after compared with before the adrenaline infusion. However, since total and cardiac noradrenaline spillovers were similar pre- and post-adrenaline, both at rest and during the handgrip, any such effect is unlikely as it would have to be closely balanced by a reduction of release/impulse in some other subdivision(s).

It cannot be excluded that there was no facilitated release of noradrenaline after the adrenaline infusion because of down-regulation of presynaptic receptors. The rather high dose of adrenaline infused could, perhaps, have resulted in persistent down-regulation of $\beta$-receptors on the sympathetic nerves, even after plasma adrenaline levels had returned to normal. If present, this might impair release of the principal neurotransmitter. Although we believe this to be unlikely, there is no direct evidence available to either confirm or exclude this possibility.

Comparison with previous results in humans

Several previous studies have studied effects of adrenaline loading of sympathetic nerves on noradrenaline plasma concentrations, haemodynamic variables and/or sympathetic effector responses in normal humans. Some studies used no stressors [35–38], in one study blood pressure and heart rate changes were monitored during 18 h of daily life activities [15], whereas several reports deal with effects of lower-body negative pressure [17–19], head-up tilt [20], mental arithmetic [16] or aerobic exercise [39]. Several studies included patients with cardiovascular disorders [20, 21, 26, 35, 39]. Results interpreted as evidence for adrenaline-induced facilitation of noradrenaline release in normal subjects were obtained by Nezu et al. [35], Fellows et al. [17] and Floras et al. [18, 19], whereas the results were negative in the study of Jern et al. [16].

In some previous studies pre-infusion data were compared with data obtained during the adrenaline infusion [20, 21]. Unfortunately, conclusions based on increased activities and/or responses or plasma noradrenaline concentrations seen during adrenaline infusions are unreliable since the effects may be secondary or coupled to the infusion-induced increase of sympathetic nerve traffic reported by Persson et al. [27] and confirmed in the present study. Data on increased plasma concentrations of noradrenaline (see above) and effector responsiveness obtained after the end of the adrenaline infusion may also be difficult to interpret. In the present study muscle sympathetic activity increased after the end of the infusion, and did not return to pre-infusion values until after approximately 20 min. This increase is probably dose-dependent, since in the study of Persson et al. [27] where the adrenaline load was greater, the nerve traffic was still more than twice the control level 30 min after the end of the infusion. Thus, the possibility exists that in some studies [17, 26] potentiated sympathetic effector responses obtained after the end of the adrenaline infusion are confounded by an increased resting sympathetic nerve traffic. The magnitude of such effects would be greatest soon after the end of the infusion but may, depending on the dose of adrenaline, still be noticeable up to 30–60 min later.

There may also be other confounding factors explaining the apparent support for adrenaline co-transmission derived from studies of effector function. For example, it cannot be excluded that adrenaline (directly or indirectly) induces long-lasting changes of the characteristics of vascular smooth muscles (e.g. contractile properties) or other effector tissues. Such effects may possibly have contributed to the results of Blankestijn et al. [15] and Floras et al. [18, 19], in which increased sympathetic nerve traffic is unlikely to have contributed.

Significance of the results

As reviewed above, adrenaline exists within sympathetic nerves, where it has largely been derived from uptake from plasma, and may be released with noradrenaline and possibly acts as a co-transmitter, augmenting the release of the major neurotransmitter [3, 4]. One theory of the pathogenesis of essential hypertension draws on these concepts [4, 7, 8], envisaging that stress-induced elevations in the plasma concentration of adrenaline may enlarge the neuronal adrenaline pool and increase neuronal adrenaline release, so as to facilitate noradrenaline
release, cardiovascular stimulation and the development of arterial hypertension [4, 7, 8]. The central questions of this hypothesis of hypertension pathogenesis are:

(1) Is circulating adrenaline taken up into sympathetic nerves?
(2) Is this adrenaline released from the sympathetic nerves?
(3) Does adrenaline released as a co-transmitter enhance noradrenaline release?
(4) Is such facilitation of noradrenaline release a cause of essential hypertension?

Understandably, it has not been easy to directly test all these elements of the hypothesis in human subjects. The first question, that of whether human sympathetic nerves extract adrenaline from plasma, can be answered in the affirmative, having been clearly demonstrated in this and previous [9, 28] studies. Regional extra-adrenal release of adrenaline has also been clearly shown in humans, for example from the sympathetic nerves of the heart during the high level of sympathetic nervous activation which accompanies aerobic exercise. But the best available evidence is that this regional adrenaline release in some contexts, such as from the heart in elderly men [11] and in patients with cardiac failure [9, 13], is from an extraneuronal source, outside sympathetic nerves. Whether extraneuronal release of adrenaline might enhance noradrenaline release from sympathetic nerves is not clear.

We were unable to detect any augmentation of noradrenaline release from the heart of healthy human volunteers, after enlarging cardiac neuronal adrenaline stores by infusing adrenaline intravenously. This negative finding contrasts with several prior studies, using less direct methods, which appeared to support the concept of adrenaline co-transmission. As discussed above this may be a misinterpretation arising from failure to recognize confounding effects of adrenaline infusions. Given these circumstances, and the fact that to-date there has been no demonstration that regional release of adrenaline, either neuronal or extraneuronal, occurs in patients with essential hypertension, the causal chain linking a stress-related elevation of plasma adrenaline concentration to the development of neurogenic human hypertension remains very incomplete.

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REFERENCES


Adrenaline is not a co-transmitter in humans


