Sympathetic nerve traffic correlates with the release of nitric oxide in humans: implications for blood pressure control

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1. Resting human sympathetic vasoconstrictor traffic displays large reproducible inter-individual differences which are similar in nerves to muscle, heart and kidney. In spite of this there is no correlation between levels of blood pressure and sympathetic traffic. To test the hypothesis that the pressor effect of the vasoconstrictor activity is counteracted by a circulating dilating factor we measured muscle nerve sympathetic activity (MSA) and an indicator of nitric oxide release (plasma nitrate) in healthy young males.

2. Sympathetic activity was recorded with the microneurographic technique in the peroneal nerve and a forearm venous plasma sample was obtained in twenty-one normotensive males aged 21–28 years. Plasma nitrate was analysed by gas chromatography and mass spectrometry.

3. There was a positive linear correlation between the plasma nitrate concentration and the strength of MSA both when the nerve activity was expressed as bursts per minute and bursts per 100 heart beats ($r = 0{\cdot}51$, $P = 0{\cdot}02$ and $r = 0{\cdot}46$, $P = 0{\cdot}04$, respectively).

4. The data suggest that the stronger the sympathetic activity the higher the release of the dilating substance, nitric oxide. This would be expected to counteract vasoconstrictor effects of the nerve traffic and thereby contribute to the lack of relationship between resting levels of MSA and blood pressure. We speculate that altered coupling between sympathetic traffic and nitric oxide release may cause abnormal peripheral resistance, e.g. in hypertension.

The strength of human muscle nerve vasoconstrictor activity (muscle sympathetic activity; MSA) displays large, reproducible interindividual differences (Sundlöf & Wallin, 1977). The differences correlate to corresponding differences in noradrenaline spillover in heart (Wallin *et al.* 1992) and kidney (Wallin, Thompson, Jennings & Esler, 1996) suggesting similar (or proportional) interindividual differences in the strength of sympathetic outflow to these tissues. Although the three vascular beds control a large part of total peripheral resistance, there is no correlation between blood pressure level and strength of MSA in normotensive subjects (Sundlöf & Wallin, 1978). In essential hypertension results vary: some studies found normal (e.g. Gudbjörnsdottir, Lönnroth, Bergman-Sverrisdottir, Wallin & Elam, 1996), others elevated MSA levels (e.g. Yamada, Miyajima, Tochikubo, Matsukawa & Ishii, 1989), but all agree that individual subjects may have high or low blood pressure regardless of MSA level. The lack of relationship between levels of blood pressure and MSA suggests that the pressor effect of the vasoconstrictor traffic is counteracted by other factor(s) coupled to the strength of MSA.

Against this background we have investigated the possibility of a systematic relationship between the strength of sympathetic vasoconstrictor traffic in muscle nerves and release of nitric oxide (NO) in healthy male subjects. To this end we recorded MSA at rest in the peroneal nerve at the level of the fibular head, and measured plasma nitrate concentration in a venous blood sample taken immediately after the nerve recording.

METHODS

The study group consisted of twenty-one healthy Caucasian male volunteers, aged 21–28 years (mean, 24·7; S.D., 1·8). All subjects gave their written, informed consent to the study, which was approved by the Human Ethics Committee of the Göteborg University.

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Recordings of sympathetic activity

Multiunit postganglionic sympathetic muscle nerve activity was recorded with the microneurographic technique in the peroneal nerve at the fibular head (Sundlöf & Wallin, 1977). The nerve signal was amplified (× 50 000), filtered (bandpass, 500–2000 Hz) and fed through a discriminator for further noise reduction and audio-monitored. A mean voltage (integrated) display was obtained by passing the original signal through a resistance–capacitance circuit (time constant, 0·1 s). During the experiments, neural activity and ECG were monitored on a storage oscilloscope, recorded on an ink-jet recorder and on videotape (Racal VStore) for subsequent analysis.

Arterial blood pressure (ABP) and ECG

Systolic and diastolic arterial blood pressures (SABP and DABP, respectively) were measured at 2 min intervals with an automatic inflated-cuff sphygmomanometer (model BP-203Y, Nippon Colin Co., Muranaka, Komaki-City, Japan). Mean arterial blood pressure (MABP) was calculated by DABP + 1/3(SABP − DABP). The average value from five measurements during the nerve registration was calculated for each individual. ECG was recorded from chest electrodes.

General procedure

Each subject visited the laboratory on two occasions with at least a 48 h interval between visits. On the first visit the subjects were informed about the study, their height and weight were measured and they were given detailed instructions about a nitrate-restricted diet, which they had to follow until the second visit. The purpose of the diet was to ensure that plasma nitrate levels reflected endogenous NO formation but not ingested nitrate/nitrite (Jungersten, Edlund, Petersson & Wennmalm, 1996). No coffee, tea or tobacco was allowed for 12 h before the second visit. On the second visit, about 2 h after a light breakfast, a microneurographic recording and measurements of blood pressure and heart rate were taken for 15 min, after which blood samples were drawn from a superficial forearm vein.

Analysis

Body mass index (BMI) was calculated from the subjects' weight and height as kilograms per metre squared (kg m^{-2}). The average blood pressure values from five consecutive measurements were calculated for each individual. Heart rate was calculated from the average R–R interval (heart period) during the microneurographic recording. Sympathetic bursts were identified by inspection of the mean voltage neurogram during a 5 min sequence in the last part of the recording period, and the amount of activity expressed as bursts per minute (bursts min^{-1}) and bursts per 100 heart beats (bursts $(100\ beats)^{-1}$). Results are expressed as means ± S.D. The relationship between variables was assessed by regression analysis. P values $< 0{\cdot}05$ were considered significant.

Plasma nitrate

Blood samples (5 ml) for the analysis of nitrate in plasma were collected in heparinized tubes. Plasma was separated immediately by centrifugation for 20 min at 1500 g and stored at −20 °C until analysis. The analysis was performed using gas chromatography and mass spectrometry. A known volume of plasma was added with a known amount of $K^{15}NO_3$ (Sigma) as internal standard. The endogenous and ^{15}N-labelled nitrate in the sample was converted to nitrobenzene by shaking a 50 µl portion for 30 min with 750 µl of benzene and 110 µl trifluoromethane-sulphonic acid (TFMS, Sigma). Before adding the plasma sample, the benzene- and TFMS-

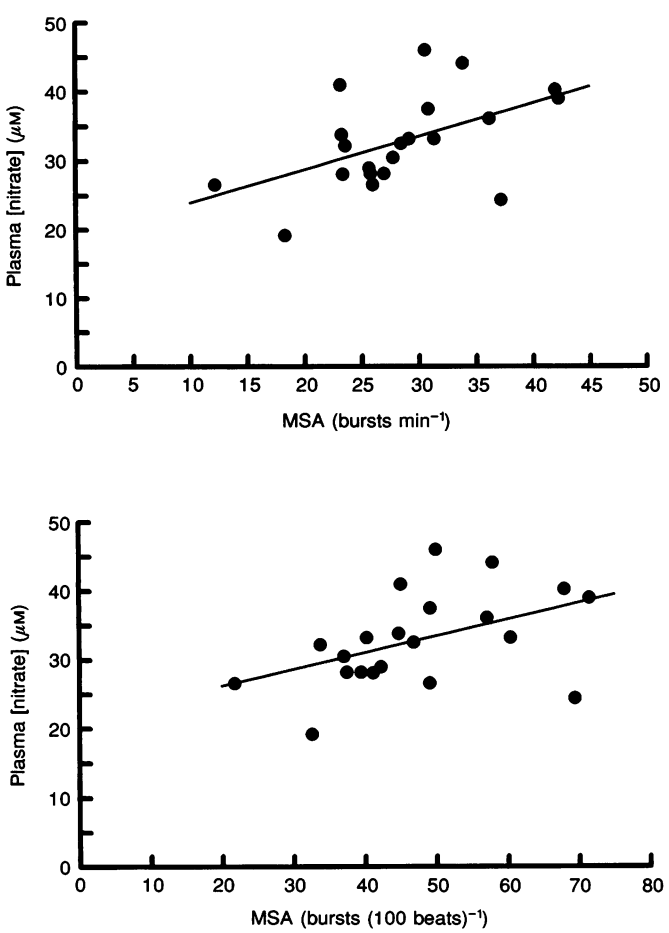

Figure 1

Linear regression plots of individual levels of resting MSA expressed as burst frequency (bursts min^{-1}, upper panel: $r = 0{\cdot}51$; $P = 0{\cdot}02$) and burst incidence (bursts $(100\ beats)^{-1}$, lower panel: $r = 0{\cdot}46$; $P = 0{\cdot}04$) *versus* plasma nitrate concentration. The graphs illustrate that a high sympathetic vasoconstrictor outflow is associated with a high production of NO and vice versa.

containing tubes were kept at −20 °C. The organic phase was separated and washed with 0·5 M $NaCO_3$. A 1 μl portion was injected into a Varian 3400 gas chromatograph equipped with a XTi-5 capillary column (Restek Corp, Bellefonte, PA, USA) operated by a temperature-regulating computer program (range, 60–260 °C). The chromatograph was connected to a Varian Saturn II mass spectrometer operated in the chemical ionization mode, using methane as the reactant gas and selective monitoring of mass equivalent (*m/e*) 124 for endogenous nitrate and *m/e* 125 for the ^{15}N-labelled internal standard. The detection limit for endogenous nitrate was 0·1 μM and the variation coefficient was 3·7%.

RESULTS

Table 1 summarizes the data obtained. All subjects were normotensive and had blood pressures below 130/80 mmHg. Their BMI was 27 kg m^{-2} or less (mean, 23 ± 2 kg m^{-2}). Plasma nitrate levels ranged from 19 to 46 μmol l^{-1}, which is similar to findings in other studies in comparable subject groups (Jungersten *et al.* 1996). The nerve traffic displayed its characteristic pulse synchronous pattern with large interindividual differences in the number of bursts (range, 22–72 bursts (100 beats)$^{-1}$ and 12–42 bursts min^{-1}) (Sundlöf & Wallin, 1977). There was a positive linear correlation between the nitrate concentration and MSA, both when the nerve activity was expressed as bursts min^{-1} and bursts (100 beats)$^{-1}$ ($r = 0·51$, $P = 0·02$ and $r = 0·46$, $P = 0·04$, respectively; Fig. 1). In contrast, no correlation was found between blood pressure (systolic, diastolic or mean arterial pressures) and MSA, or between blood pressure levels and plasma nitrate concentrations (Fig. 2).

Table 1. Data on plasma nitrate, haemodynamic and neural variables

	Mean	S.D.	Range
SABP (mmHg)	114·1	7·0	102·2–129·2
DABP (mmHg)	70·2	5·5	60·2–78·6
HR (beats min^{-1})	60·4	7·1	51·5–75·0
MSA (bursts min^{-1})	28·5	7·3	12·2–42·3
MSA (bursts (100 beats)$^{-1}$)	47·4	12·9	21·8–71·5
[Nitrate] (μM)	32·8	6·8	19·2–46·0

DISCUSSION

The metabolism and kinetics of NO reaching the blood has been studied previously. In humans, after inhalation of ^{15}NO, ^{15}N-labelled nitrate was found to be a major plasma metabolite, and $^{15}NO_3^-$ excreted in urine accounted for 75%

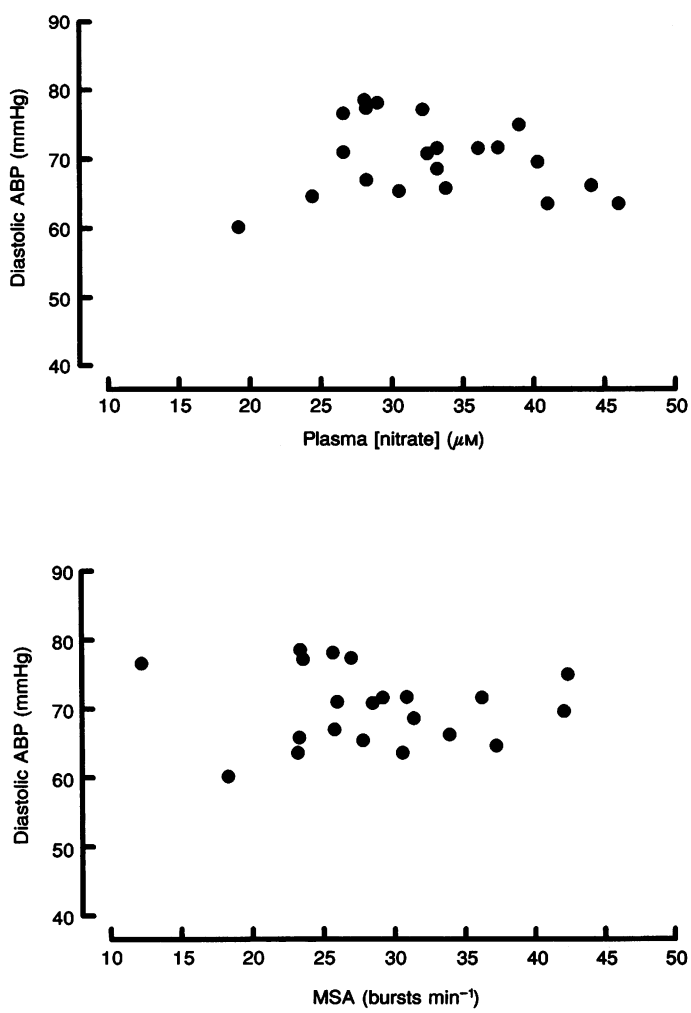

Figure 2

The plots show the lack of relationship between plasma nitrate level and diastolic blood pressure (upper panel: $r = -0·13$; $P = 0·57$) or between MSA and diastolic blood pressure (lower panel: $r = -0·08$; $P = 0·74$).

of the amount of isotope inhaled (Nathorst-Westfelt, Benthin, Lundin, Stenqvist & Wennmalm, 1995). The NO released into the blood may also form other metabolites such as *S*-nitrosylated proteins (Stamler *et al.* 1992), but being the main stable metabolite, nitrate has been adopted as an index of the overall formation of NO *in vivo* (Winlaw, Smythe, Keogh, Schyvens, Spratt & Macdonald, 1994). When using plasma nitrate concentration as an index of the endogenous formation of NO, confounding factors as well as the kinetics of nitrate *in vivo* have to be taken into account. It has been demonstrated that 48 h of nitrate/nitrite-restricted diet (no vegetables or ham) is an absolute requirement if plasma nitrate is to reflect the endogenous formation of NO (Jungersten *et al.* 1996). In addition, to avoid the influence on nitrate from exercise-induced NO formation (Jungersten, Ambring, Wall & Wennmalm, 1997) all subjects were instructed to refrain from heavy physical exercise during the diet period. Since these factors were taken into account, we believe that the presently observed nitrate levels reflect the endogenous formation of NO at rest.

Previous studies have shown that healthy subjects with marked differences in resting MSA may have similar blood pressure levels (see above). Several explanations are possible: (1) strong vasoconstrictor activity in nerves to one vascular bed may be coupled to weak activity in nerves to other beds (sympathetic differentiation); (2) a high total peripheral vascular resistance induced by strong nerve traffic may be balanced by a low cardiac output; and (3) the vasoconstrictor effect of strong nerve traffic may be counteracted by a low transmitter release/impulse, by a low number/sensitivity of vascular adrenergic receptors, or by a high level of a circulating dilating factor. The positive correlations found between MSA and noradrenaline spillover in the heart (Wallin *et al.* 1992) and the kidney (Wallin *et al.* 1996) suggest that MSA reflects sympathetic vasoconstrictor traffic to at least three vascular beds controlling a large part of total peripheral resistance. This makes the first alternative less likely. No previous data from human nerve recordings relate to the other alternatives but the present results suggest that the release of the dilating substance NO is coupled quantitatively to the strength of MSA in healthy young males. This would be expected to counteract the vasoconstrictor effects of the sympathetic traffic and would therefore contribute to the lack of relationship between resting levels of MSA and blood pressure. Several other circulating factors may have dilating or constricting effects on vascular smooth muscles (i.e. endothelin, prostacyclin and angiotensin) but whether their concentrations are related systematically to the strength of sympathetic nerve traffic is unknown.

Several explanations for the coupling between MSA and NO are possible. Firstly, NO synthase has been found in sympathetic nerves, which suggests that NO may be released as a cotransmitter (Dun, Dun, Wu & Förstermann, 1993; Modin, Weitzberg, Hökfelt & Lundberg, 1994). However, these findings relate primarily to preganglionic neurones and, in addition, contractile responses to transmural electrical stimulation in human saphenous veins were unaffected by application of NO synthase inhibitor and removal of the endothelium (Fabi, Argiolas, Chiavrelli & Del Basso, 1996). Thus, postganglionic sympathetic NO release may not be the source of the high plasma nitrate associated with high MSA. Secondly, endothelial cells have adrenergic (Miller & Vanhoutte, 1985) and other receptors which can stimulate NO release (Cocks & Angus, 1983; Fabi *et al.* 1996). It is known that MSA correlates positively with plasma concentrations of noradrenaline (Wallin, Sundlöf, Eriksson, Dominiak, Grobecker & Lindblad, 1981). Thus, it may be circulating noradrenaline that stimulates NO release from the endothelium, thereby providing an apparent (indirect) coupling between MSA and endogenous NO release. Thirdly, vasoconstriction may increase shear stress on the vascular wall, which in turn, increases NO release from vascular endothelial cells (Miller & Burnett, 1992). Whether or not this mechanism contributes to the present results is also unclear since sympathetically mediated mesenteric vasoconstriction has been found to decrease rather than increase shear stress (Nase & Boeghold, 1996).

One way to establish further the relationship between sympathetic vasoconstrictor activity and NO formation might be to measure plasma nitrate levels during manoeuvres known to increase sympathetic nerve traffic. Unfortunately, however, nitrate concentrations are not well suited for determination of transient changes of NO formation. Based on the urinary excretion at rest (Jungersten *et al.* 1997), the overall formation rate of nitrate in the body is about 1 μmol min^{-1} and the distribution volume is 30% of body weight (Jungersten *et al.* 1996). Accordingly, a doubling of overall NO formation during 30 min would increase the plasma concentration by at most 1–2 μmol l^{-1}, i.e. a 3–6% increase of the basal nitrate level (assuming immediate conversion to nitrate and complete distribution in its distribution volume). Thus, a sufficiently large, long-lasting increase of sympathetic nerve traffic with associated changes in plasma nitrate would be difficult or impossible to achieve in normal subjects. For this reason no such manoeuvres were performed in the present study.

A more appropriate way to investigate the relationship between vasoconstrictor nerve activity and NO formation may be to study states of chronically increased sympathetic nerve traffic. In agreement with this, the marked increase in resting MSA seen in congestive heart failure (Leimbach, Wallin, Victor, Aylward, Sundlöf & Mark, 1986) has been shown to be associated with elevated plasma nitrate levels (Winlaw *et al.* 1994). The presently observed relationship between vasoconstrictor nerve traffic and NO release raises the possibility that abnormalities in peripheral vascular resistance may be explained by factors that distort this coupling. For instance, an increased total peripheral resistance leading to hypertension could arise if the balance is tilted towards vasoconstrictor dominance, either via an excessive MSA without concomitant increase of NO release,

or via reduction of NO availability, in the face of an unchanged MSA. Such variation of mechanisms may explain why both an unchanged (Gudbjörnsdottir *et al.* 1996) and increased (Yamada *et al.* 1989) MSA has been found in essential hypertension.

In summary, we have found a positive linear correlation in young heathy males between the plasma nitrate concentration and the strength of muscle nerve sympathetic activity, suggesting that the stronger the sympathetic activity the higher the release of the dilating substance NO. This should counteract vasoconstrictor effects of the nerve traffic and thereby contribute to the lack of relationship between resting levels of MSA and blood pressure.

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