

Human cardiovascular variability, baroreflex and hormonal adaptations to a blood donation

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A B S T R A C T

1. We studied cardiovascular variability, baroreflex and blood volume regulating hormones to determine the relative roles of autonomic regulation and hormones during blood donation.
2. The sympathetic response was studied by measuring the R–R interval and systolic blood pressure variability using coarse graining spectral analysis in eight blood donors. Beat-by-beat R–R intervals and blood pressure were recorded for 20 min before and 5 min after a whole-blood donation of 480 ± 10 ml (about 7 ml/kg of blood volume, over 4 min). Plasma catecholamines, vasopressin, atrial natriuretic peptide, endothelin, active renin, osmolality, Na^+ , K^+ , haemoglobin and haematocrit were measured just before and after blood withdrawal.
3. Blood donation led to increases in the plasma catecholamines (adrenaline, 21 ± 2 versus 35 ± 3 pg/ml; noradrenaline, 229 ± 26 versus 323 ± 37 pg/ml; dopamine, 34 ± 3 versus 66 ± 9 pg/ml) and in systolic blood pressure (130 ± 6 versus 140 ± 5 mmHg). These changes were independent of ionic or slow endocrine mechanisms. Heart rate, cardiovascular variability and the spontaneous baroreflex sensitivity did not change despite the increase in blood pressure and catecholamines. Thus the peripheral vascular control was probably involved.
4. We conclude that the absence of any change in heart rate usually observed during non-hypotensive hypovolaemic stress is probably due to the sympathetic activation being counter-balanced by the high supine vagal tone at the heart and not to the heterogeneous nature of the sympathetic neural response or to changes in sympathetic and parasympathetic activity without any change in autonomic balance.

INTRODUCTION

Blood pressure depends in part on the blood volume. Despite large variations in liquid input and output, the blood pressure remains well regulated over a small range. Short-term regulation is due to reflexes and mid- and long-term regulation relies on endocrine mechanisms. The long-term regulation of blood pressure by controlling blood volume has been extensively studied

because of its role in hypertension. Short-term regulation has also been studied during postural fluid redistribution and after the loss of large volumes of blood, which lead to a change in blood pressure. These studies can help clarify the changes that occur in haemorrhagic shock. There have been fewer studies on short-term adaptation to a non-hypotensive acute drop in blood volume, although it is common during mild haemorrhage or blood donation. Clinicians are sometimes confronted with (pre-) syncopal

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Abbreviation: CGSA, coarse graining spectral analysis.

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symptoms in these situations. There is also a 10% decrease of blood volume during space flights lasting several days and in ground-based simulated weightlessness, after which subjects may experience orthostatic intolerance when standing [1]. A better understanding of the relationships between changes in blood volume and the stability of the cardiovascular system could improve the prediction and prevention of orthostatic intolerance. The short-term consequences of a mild drop in blood volume are still unclear. The (low-pressure) cardiopulmonary baroreceptors are probably unloaded. The central venous pressure [2], cardiac filling pressure [3] and left atrial volume [3] all decrease, and the lack of any decrease in blood pressure without a change in heart rate suggests that the (high pressure) arterial baroreceptors are not involved [4]. Several explanations have been given for the adaptation to a specific unloading of cardiopulmonary baroreceptors [5] without any definitive answer. This study was carried out to verify that a donation of 10% of the donor's blood volume caused a rapid resetting of the baroreflex and that the lack of a consistent change in heart rate during this protocol could result from the heterogeneous sensitivity of peripheral effectors to a sympathetic stimulation.

MATERIALS AND METHODS

Subjects

A group of eight healthy subjects [seven men and one woman; age, 33 ± 3 (range 25–45) years; height, 175 ± 2 (167–181) cm; weight, 69 ± 3 (56–82) kg], volunteered for this study. They were regular blood donors from the hospital staff and were familiar with the blood donation room and procedure. None suffered from any chronic disease, had any recent acute disease, or was taking any medication. The subjects were given a complete description of experimental procedure and they signed a consent form. No cigarettes or caffeine-containing beverages were allowed for 12 h before the experiment. The data collection procedure was approved by the Comité Consultatif de Protection des Personnes Midi-Pyrénées I.

Experiment

The experiment took place in the blood donation room of the Etablissement de Transfusion Sanguine de Nîmes. Two hours after a light lunch the subjects were interviewed and examined as part of the regular medical check-up before each blood donation. Their resting heart rate and manometer blood pressure were measured. The subjects then laid in the comfortable blood donation chair for 10 min during which time ECG electrodes and a finger blood pressure cuff were fitted. R–R intervals and systolic blood pressure were then recorded on a beat-to-beat basis over 15–25 min. A 20-ml blood sample was

taken by direct venous puncture after this first recording. A trained phlebotomist performed all venepunctures. The regular blood donation was collected in a standard blood collection bag over about 4–5 min. The subjects did not perform hand grip during the phlebotomy to avoid any influence of isometric exercise on the sympathetic response. A second blood sample was obtained after clamping directly from the tubing for the regular medical screening and 20 ml for the experiment. The second beat-by-beat recording was then made after stabilization of heart rate and blood pressure (about 5 min after the end of the donation). At the end of the second recording the subject was allowed to stand and go for a snack.

Blood sample analysis

Each blood sample was divided in two; 5 ml was mixed with EDTA and 15 ml was placed in heparinized tubes. The haematocrit and haemoglobin were immediately determined in the EDTA samples using a cell counter (Onyx, Coulter, FL, U.S.A.). The heparinized samples were immediately centrifuged at 1500 g for 10 min at 4 °C. The plasma was stored at –40 °C until assayed. Plasma sodium and potassium (IL Flame photometer, Milan, Italy) and osmolality (Fisk OR Osmometer, MA, U.S.A.) were measured. The plasma concentrations of catecholamines (adrenaline, noradrenaline and dopamine) were measured by HPLC with electrochemical detection [6]. Plasma vasopressin [7], atrial natriuretic peptide [8], endothelin [9] and active renin (Renin IRMA, Pasteur Kit, ref. 79897) were determined by radioimmunoassay.

Recordings

Two-lead surface ECG and Finapres 2300 (Ohmeda, Englewood, CO, U.S.A.) blood pressure signals were recorded from each subject before and after blood donation. Recordings were long enough to obtain at least 1024 heartbeats (15–25 min). A peak detection circuit was used to discriminate the R-wave from the ECG. The impulse train was processed in real-time on a personal computer via an analogue-to-digital converter (DAS-16G, Keithley-Metrabyte, Taunton, MA, U.S.A.) at a sampling frequency of 1000 Hz and a resolution of 12 bits. The beat-by-beat R–R intervals and systolic and diastolic blood pressures were stored for later analysis. Each series was searched for abnormal values (defined as values 25% greater or less than the preceding value) before analysis. Very few were identified (< 1%). Abnormal intervals were typically due to a missed beat, or to triggering the T-wave as well as the QRS complex. A beat was inserted when one was missed, while the two short interval values were deleted when the T-wave was triggered and a beat was inserted by interpolation. The filtered R–R interval and systolic blood pressure data were then aligned sequentially to obtain equally spaced

samples of R–R interval series and systolic blood pressure series [10]. The mean arterial blood pressure (2/3 of diastolic blood pressure plus 1/3 of systolic blood pressure) and pulse arterial blood pressure (systolic blood pressure minus the preceding diastolic blood pressure) were computed. The recording conditions were strictly controlled to approach stationarity. Each subject was familiar with the experiment room and was made familiar with the equipment before the first recording. Subjects were asked to avoid any movement, to breathe quietly, and to be as relaxed as possible but not to sleep. The experiment room was quiet and air-conditioned with no bright light.

Time series analysis

The means and S.D.s of the R–R interval and systolic and diastolic blood pressure were obtained for each recording. The fractal components of R–R interval variability and of systolic blood pressure variability were analysed by subjecting the 1024-data-point time series to coarse graining spectral analysis (CGSA) [11]. CGSA discriminated fractal random walks from simple harmonic motion based on characteristics that the original and rescaled (coarse grained) time series had random phase relationships only for fractal signals. Two rescaled versions of the original time series were obtained by sampling every second value or holding each value for two sample points. The fractal component was plotted in a log-power versus log-frequency plane and the spectral exponent (β) was estimated as the slope of the linear regression of this plot from 2.5% of Nyquist frequency to 0.3 Hz or higher, if the plot was still linear at higher frequencies [12]. The CGSA method also provided a power spectrum for the harmonic (or regular oscillatory) components which have been used to evaluate the cardiac autonomic outflow [11]. Harmonic spectral components were analysed to determine the power in the low-frequency (0.0–0.15 Hz), high-frequency (0.15–0.50 Hz) and total (0.0–0.50 Hz) domains of heart rate variability and of blood pressure variability spectral analysis. The spectra were only divided into two frequency domains because autonomic regulation of the heart depends only on the two branches of the autonomic nervous system and because CGSA allows measures of the harmonics over the whole frequency range. The frequency domain measures determined were those previously recommended [13].

Spontaneous baroreflex

Beat-by-beat data for the R–R interval and systolic blood pressure were examined for the occurrence of spontaneous baroreflex sequences [14]. Three or more consecutive heartbeats in which changes in systolic pressure of at least 1 mmHg were accompanied by directionally similar changes in R–R interval of at least 1 ms were considered to be baroreflex responses. A linear regression

was fitted to each individual sequence for a subject during the data analysis period. The mean response slope was taken as the average of these individual regressions. Parlow et al. [15] showed that the same methods provide values of baroreflex sensitivity similar to those obtained with the drug-induced method. We also computed the gain in the mid-frequency band (0.07–0.14 Hz) for systolic blood pressure and R–R intervals by cross-spectral analysis [16]. The spontaneous baroreflex and the gain in the mid-frequency band were computed three times for each subject, in the last 5 min of the pre-donation recording, and in the first and last 5 min of the post-donation recording.

Statistics

Values are expressed as means \pm S.E.M. The statistical comparison of values before and after blood donation was performed using a Wilcoxon test for paired data for each of the blood and cardiovascular parameters. The difference of spontaneous baroreflex and gain in the mid-frequency band between the three determinations was assessed by the Kruskal–Wallis test for paired data. The significance level was set at $P \leq 0.05$.

RESULTS

The total blood volume taken was 480 ± 10 ml (about 7 ml/kg), including the experimental and screening samples. Blood donation was well tolerated and no subject showed any vagal sign, (pre-) syncopal symptom or orthostatic intolerance when standing 30 min after donation.

Blood samples (Table I)

The plasma adrenaline, noradrenaline and dopamine concentrations were slightly but significantly higher after

Table I Blood and plasma assay results

The plasma parameters before and after blood donation. NS, not significant.

	Before	After	<i>P</i> value
Osmolality (mosmol/kg H ₂ O)	301 \pm 2	302 \pm 3	NS
Vasopressin (pg/ml)	2.9 \pm 0.6	3.0 \pm 0.7	NS
Atrial natriuretic peptide (pg/ml)	20.8 \pm 3.0	22.8 \pm 3.1	NS
Endothelin (pg/ml)	5.9 \pm 1.0	5.5 \pm 1.0	NS
Active renin (pg/ml)	10.6 \pm 2.3	10.1 \pm 2.4	NS
Haemoglobin (g/l)	142 \pm 3	142 \pm 3	NS
Haematocrit (%)	42.3 \pm 0.9	42.1 \pm 0.9	NS
Na ⁺ (meq/l)	136.3 \pm 0.6	136.2 \pm 0.7	NS
K ⁺ (meq/l)	4.1 \pm 0.2	3.9 \pm 0.1	NS
Adrenaline (pg/ml)	21 \pm 2	35 \pm 3	<i>P</i> \leq 0.05
Noradrenaline (pg/ml)	229 \pm 26	323 \pm 37	<i>P</i> \leq 0.05
Dopamine (pg/ml)	34 \pm 3	66 \pm 9	<i>P</i> \leq 0.05

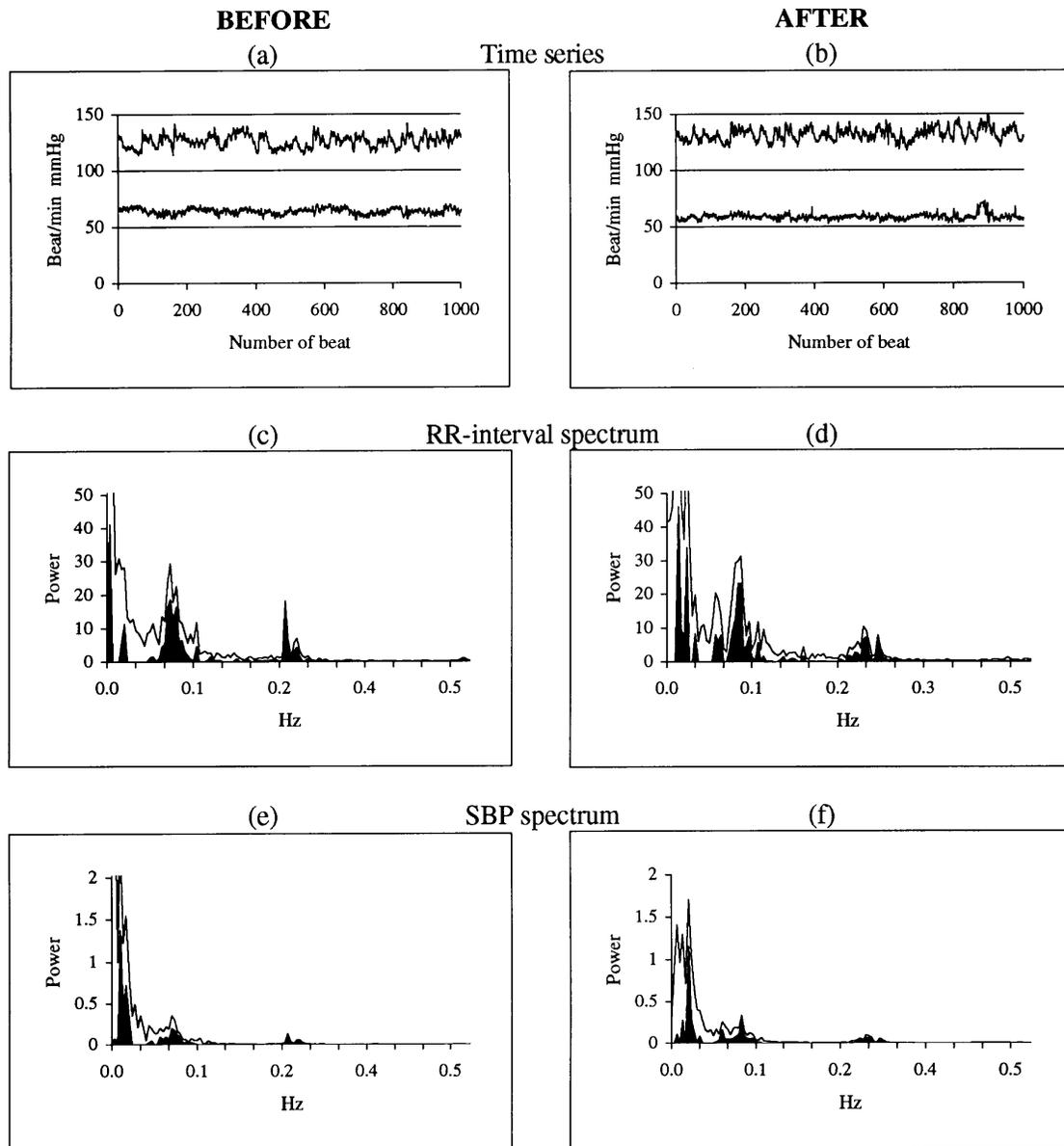


Figure 1 Cardiovascular recordings and their spectral analysis of one subject before and after blood donation

Panels (a) and (b) represent systolic blood pressure (upper curves) and heart rate series (lower curves; heart rate is shown instead of R–R intervals for scale convenience). The spectral analysis diagrams of R–R interval (c and d) and of systolic blood pressure (e and f) show the regular fast Fourier transform (thin spectrum) and the coarse graining spectral analysis (dark spectrum) before (c and e) and after (d and f) a blood donation. The difference between thin and dark spectrum corresponds to the fractal noise contained in the series.

blood donation. The other blood parameters measured were not altered by the blood donation.

Cardiovascular parameters

Figure 1 presents the cardiovascular recordings of one subject and their corresponding spectral analysis before and after blood donation. Blood donation led to a slight but significant increase in systolic and mean blood pressures. Heart rate, pulse and diastolic blood pressure were not altered (Table 2). Spectral analysis of R–R interval variability showed no alteration of the autonomic

influence on the heart. Blood donation induced a significant increase in the high-frequency component of blood pressure variability (Table 3). This effect disappeared when the spectral components were normalized. The spectral exponent of R–R interval variability tended to increase after blood donation but it did not reach a significant level. Fractal analysis of blood pressure variability did not show any difference either for the percentage of fractal or for the spectral exponent (β). The spontaneous baroreflex was similar at the three determinations (just before: 11.9 ± 1.4 ms/mmHg, just after:

Table 2 Cardiovascular parameters

The cardiovascular parameters before and after blood donation. BP, blood pressure; NS, not significant.

	Before	After	<i>P</i> value
Systolic BP (mmHg)	130.3 ± 6.1	140.4 ± 5.1	<i>P</i> ≤ 0.05
Diastolic BP (mmHg)	69.2 ± 5.5	74.6 ± 4.2	NS
Mean BP (mmHg)	89.9 ± 5.9	97.1 ± 4.6	<i>P</i> ≤ 0.05
Pulse BP (mmHg)	62.3 ± 3.0	67.4 ± 2.5	NS
R–R interval (ms)	841 ± 34	877 ± 37	NS

11.8 ± 2.8 ms/mmHg, and about 15 min after blood donation: 11.1 ± 2.1 ms/mmHg). The number of spontaneous events was never less than 17. The gain of the mid-frequency band tended to increase between the three determinations but it did not reach a significant level (just before: 11.3 ± 1.4, just after: 12.0 ± 1.6, and about 15 min after blood donation: 10.6 ± 1.3 ms/mmHg).

DISCUSSION

Blood donation can activate the sympathetic nervous system since plasma catecholamines and systolic blood pressure increased. Neither heart rate nor heart rate variability were influenced by the blood donation. The blood pressure variability spectral analysis was not clearly altered. This rapid adaptation does not seem to have involved any endocrine mechanisms [4,17].

Heart rate and blood pressure are both very susceptible to external disturbance or stress. The recording conditions were therefore strictly controlled to ensure that the variability was due to the intrinsic cardiovascular regulatory system (see Materials and Methods section). The rise in the plasma concentration of catecholamines and the increase in blood pressure suggest that our

protocol led to an overall sympathetic activation. Much of neuronally released noradrenaline is metabolized locally or sequestered by neuronal re-uptake. Thus an increase in plasma noradrenaline is not necessarily a good indicator of sympathetic activation. However, Rea et al. [2] confirmed the overall sympathetic activation by recording muscle sympathetic nerve activity during mild changes in blood volume produced by haemorrhage. The significant increase in the high-frequency component of systolic blood pressure variability is not concordant with a sympathetic activation. The normalized high-frequency component of systolic blood pressure variability did not change. Heart rate was not altered, despite the overall sympathetic activation. This profile has not always been observed after blood donation or a mild drop in blood volume [2,17–20]. Our results also contrast with those observed after a plasmaphoresis [21]. This procedure led to an increase in the total power and in the absolute power of the low-frequency component of blood pressure variability [21]. The plasmaphoresis was completed within 50 min and the procedure is very different from a regular blood donation. The response of a reflex system could depend on the experimental procedure used. The time taken for the decrease in blood volume could be an important factor [22]; in our study it was very fast (about 4–5 min). The species studied is probably also an important factor. Some experimental studies on haemorrhage have been performed in animals. Persson [4] pointed out the species specificity of arterial and cardiopulmonary baroreceptor interactions. These points explain the differences in the responses observed during a mild drop in blood volume.

The lack of change in the haematocrit or blood haemoglobin means that the blood loss was not offset by any transcapillary fluid shift by the time the second blood sample was taken. The endocrine system was not activated in response to the experimental stimulus, which is logical considering that hormones are mild, long-term

Table 3 Spectral analysis

Results of R–R interval and systolic blood pressure CGSA before and after blood donation. LF, low-frequency power; HF, high-frequency power. Statistical significance: **P* ≤ 0.05.

	R–R interval		Systolic blood pressure	
	Before	After	Before	After
Total power	1193 ± 165 (ms ² /Hz)	1373 ± 270 (ms ² /Hz)	23.34 ± 8.41 (mmHg ² /Hz)	27.83 ± 8.77 (mmHg ² /Hz)
LF	163.07 ± 34.46 (ms ² /Hz)	194.97 ± 29.90 (ms ² /Hz)	5.42 ± 2.98 (mmHg ² /Hz)	3.90 ± 1.26 (mmHg ² /Hz)
LF/Total power	0.14 ± 0.02	0.16 ± 0.03	0.19 ± 0.04	0.15 ± 0.03
HF	129.26 ± 50.71 (ms ² /Hz)	148.84 ± 67.41 (ms ² /Hz)	0.30 ± 0.12 (mmHg ² /Hz)	0.47 ± 0.17 (mmHg ² /Hz)*
HF/Total power	0.09 ± 0.03	0.09 ± 0.02	0.02 ± 0.01	0.03 ± 0.01
LF/HF	5.1 ± 3.1	11.7 ± 6.0	55.90 ± 36.65	32.38 ± 25.57
β	1.098 ± 0.120	1.315 ± 0.184	2.025 ± 0.113	1.896 ± 0.146
% Fractal	77 ± 3%	76 ± 2%	79 ± 3%	82 ± 3%

regulatory factors. Renin secretion depends on the rate of haemorrhage [22], but non-hypotensive bleeding does not seem to cause a rapid change in renin or vasopressin [22]. Several things could lead to sympathetic activation. Despite the time course described by Robertson [22], where the increase in renin began earlier than the increase in plasma catecholamines, the sympathetic activation that occurred in our study was not triggered by active renin, as it did not change. Sympathetic activation could be a central response to the needle stress or to the emotional experience of blood donation. The response induced by haemorrhage in chronically instrumented animals should not depend on direct central influences which often interact in human bleeding. It has become common practice to draw blood samples for plasma catecholamines through an indwelling intravenous catheter or needle to avoid the needle stress in humans [23]. However, the conditions of the direct venepuncture for the control sampling are closer to the blood donation procedure where the indwelling catheter or needle are avoided (French blood donation regulation). Our study was performed on experienced blood donors who were also hospital staff and the level of catecholamines before the blood donation were in accordance with the standard values of our laboratory. The sympathetic activation that occurred in our study could be due to baroreceptors unloading. A mild drop in blood volume is usually considered to be a specific stimulus of low pressure cardiopulmonary baroreceptors [4]. However, non-hypotensive haemorrhage decreases the activity of aortic baroreceptors, regardless of whether there is a decrease in the mean aortic pressure [19]. Our protocol with its mild drop in blood volume and increased systolic and mean blood pressures probably did not involve arterial baroreceptors. Arterial baroreceptors should act in the opposite way, to decrease heart rate in response to the increased blood pressure. Oren et al. [3] found that unloading the left atrium elicited a reflex sympatho-excitatory response with a low level of lower body negative pressure. Rea et al. [2] showed that the sympathetic responses to low-level lower body negative pressure and non-hypotensive haemorrhage are similar in humans. Low-pressure baroreceptor unloading is also observed during gravitational stress (standing) and prolonged head-down bed-rest in which an alteration of the fractal component of heart rate variability was reported [12,24]. The lack of fractal alteration after blood donation demonstrates that the fractal changes observed during bed rest or during standing are not related to the blood volume changes.

Whatever the origin of the sympathetic activation induced by our protocol, stress or low-pressure receptor unloading, heart rate is not altered. This surprising dissociation of blood pressure and heart rate occurred despite the close link between these two variables. The lack of alteration of spontaneous baroreflex sensitivity

and heart rate with a shift in blood pressure indicates that the baroreflex is rapidly reset. This resetting could occur in 90 s [25]. Several factors may reset the baroreflex such as changes in the vascular wall, in ions or paracrine, endocrine or central influences. Chronic resetting depends upon several of these factors [26]. The fast baroreflex resetting observed here could occur without ionic or endocrine alterations. Vascular wall alterations were probably involved because there was probably an increase in peripheral vascular resistance [4], which increased blood pressure, but did not change heart rate. This vasoconstriction was probably not due to a slow endocrine change. Specific stimulation of low-pressure baroreceptors generally leads to a peripheral sympathetic response without any effect on the heart. The absence of change in heart rate during mild hypovolaemic stress could result from the absence of a cardiac effect due to the heterogeneous nature of sympathetic neural responses, offsetting changes in the mean vagal and sympathetic cardiac activity, or changes in the sympathetic or vagal modulation (or both) of heart rate without a change in mean autonomic tone [5]. The increase in plasma catecholamines indicates an overall sympathetic activation. The lack of any increase in the R-R interval in response to the increase in blood pressure could signal cardiac activation. The normalized low-frequency component of heart rate variability which is influenced by the sympathetic activity also doubled although this increase was not significant. Such an increase is moderate when compared with that observed during tilt or mental stress [27]. R-R interval variability and mean heart rate were not altered, indicating that this slight sympathetic stimulation has no effect on the chronotropism, but it does not necessarily mean that it has no effect on the heart. Levy and Zieske [28] showed that the effect of the same sympathetic stimulation on heart rate depended on the parasympathetic tone, which was not the case for the effect on heart conduction (dromotropism). Thus a slight sympathetic activation in the context of a high parasympathetic tone will not affect the heart rate but will affect the vascular bed, which is only influenced by the sympathetic branch of the autonomic nervous system. Taken together, these results suggest that a small activation of the sympathetic nervous system which leads to a small increase in blood pressure has no effect on heart rate because its effect is masked by the supine high parasympathetic tone. The hypothesis which implies changes in the sympathetic and parasympathetic influences on the heart without any change in the autonomic balance is not supported by our results. There appears to be no significant change in the spectral analysis of R-R interval variability.

In conclusion, the slight sympathetic activation that results from a blood donation leads to a rapid resetting of the baroreflex which is independent of any ionic or endocrine influences. The absence of any change in the

heart rate or in its dynamics during this stimulus is probably due to the non-linear interaction between sympathetic and parasympathetic influences on the sinoatrial node. Thus the lack of change in chronotropism during blood donation could result from the heterogeneous sensitivity of the peripheral effectors to sympathetic stimulation. A study of the overall autonomic influences on the heart during this kind of response should be very informative.

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