JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2013, 64, 5, 649-655
www.jpp.krakow.pl

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SPLEEN VOLUME CHANGES DURING ADRENERGIC STIMULATION WITH LOW DOSES OF EPINEPHRINE

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It is generally accepted that the spleen contraction is a consequence of humoral stimulation but recent data suggest a role of neural mechanisms. This study tested the hypothesis that the reduction in spleen size in response to low dose epinephrine infusion is a consequence of neurally mediated unloading of baroreceptors. Continuous ultrasonic measurements of spleen volume in response to intravenous infusion of low doses of epinephrine (0.06 μg/kg/min for 6 minutes, followed 0.12 μg/kg/min for 3 minutes) were performed with simultaneous continuous noninvasive measurements of cardiovascular parameters in thirteen subjects. In subgroup of six subjects we also continuously measured muscle sympathetic nerve activity (MSNA) as an index of peripheral sympathetic activation. Significant spleen contraction (~30%, p=0.008) was observed early after the onset of epinephrine infusion and was preceded by a decrease in total peripheral resistance (41%, p=0.001) and mean arterial pressure (6.2%, p=0.02) and an increase in heart rate (27%, p=0.001) and total MSNA (120%, p=0.02). Our results demonstrate rapid spleen contraction induced by low-dose epinephrine infusion in conditions of decreased blood pressure and increased MSNA suggesting that the spleen may represent a constitutive part of the sympathetic nervous system under stressful situations.

Key words: *spleen, baroreceptors, epinephrine, muscle sympathetic nerve activity, mean arterial pressure, alpha-adrenoreceptors, beta- adrenoreceptors*

INTRODUCTION

Spleen contraction occurs in response to diverse stimuli both in mammals and humans (1-4). Human spleen's innervation originates in the superior mesenteric/coelic ganglion with nerve fibers entering the spleen associated with the splenic artery. The splenic nerve contains approximately 98% sympathetic nerve fibers with the greatest density within the central artery of the white pulp and associated periarterial lymphatic sheath (5, 6). Using immunohistochemical staining contractile proteins were identified not only within the walls of arteries, veins, splenic capsule and trabeculae, but also within the reticular cells of the white pulp and sinus lining cells of the red pulp of the spleen (7). These findings suggest that human spleen could be capable of contracting and regulating its volume through internal neural network. It is generally accepted that splenic contraction is a passive rather than an active process, and it is consequence of humoral stimulation mediated *via* adrenoreceptors (α_1, α_2, β_1, and β_2) located in the splenic capsule and parenchyma (1, 8). It was reported that activation of β-adrenoreceptors on the spleen causes its expansion while activation of α-adrenoreceptors causes its contraction (9-11). However, recent studies have observed extremely fast spleen contraction where a decrease in spleen volume occurred at the very beginning of the apnea (used as stimulus for spleen contraction) (12, 13) with the increase in heart rate and a decrease in mean arterial pressure (13, 14), suggesting that the mechanism of contraction may be neurally mediated by unloading of baroreceptors.

To determine the relationship between the central sympathetic system and spleen contraction we investigated changes in spleen volume after a low dose of epinephrine intravenous infusion in healthy young men. Theoretically, it is possible that both direct and indirect effects of epinephrine infusion exist. For example, epinephrine infusion, by altering central hemodynamics, may affect sympathetic discharge. This, in turn, may interfere with direct effects of epinephrine. We first planned to steadily increase the dose of epinephrine until we accomplish α-adrenoceptor stimulation and spleen contraction. However, unexpectedly, we observed fast and massive spleen contraction by the second minute of low dose epinephrine infusion with a concomitant decrease in mean arterial pressure and total peripheral resistance. These findings raised an idea that a decrease in mean arterial pressure induced by low doses of epinephrine can cause an increase in sympathetic nerve activity and thereby a decrease in spleen volume. Therefore, we monitored muscle sympathetic activity (MSNA), as an index of peripheral sympathetic activation, to estimate a role of reflex-mediated sympathetic nerve activity in the splenic contraction with low doses of epinephrine.

MATERIAL AND METHODS

All experimental procedures were performed in accordance with the Declaration of Helsinki on the treatment of human subjects and were approved by the ethical committee of the

University of Split School of Medicine. Each method and potential risks were explained to the participants in detail and they gave their written informed consent before the experiment.

Participants

Thirteen healthy male subjects, aged 25.9±2.5 years (mean ±S.D.) participated in the study. The average weight was 90±10 kg (mean ±S.D.) and height 188.7±5 cm (mean ±S.D.). All were healthy non-smokers.

Protocol

All experiments were carried out in the acclimatized environment in the morning hours with constant temperature of 22–25°C and humidity of 25–45%. One participant was tested each day. They arrived to the laboratory 45 minutes before the start of the experiment for acclimatization and detailed explanation of the procedures. All infusions were performed after an overnight fast, including abstinence from caffeine and tobacco. The subjects were supine throughout infusion. Intravenous catheter was inserted into antecubital vein 30 min before the infusion of epinephrine and the subjects rested in a supine position. An intravenous infusion of epinephrine (1% adrenaline - HCl, Park-Davis), was prepared by diluting 1 mg epinephrine (an ampoule of 1 ml contains 1 mg of epinephrine) in 250 ml of 0.9% NaCl, and infused with starting dose of 0.06 μg/kg/min over a period of 6 minutes, and at the dose of 0.12 μg/kg/min over the following 3 minutes.

Cardiovascular measurements

Continuous, noninvasive monitoring of the heart rate (HR) and blood pressure (Finometer, Finapress Medical Systems, Arnhem, Netherlands) was obtained from the middle finger of the non-dominant hand. The photoplethysmographic values of diastolic and systolic blood pressures were gauged using the mercury sphigmomanometer. The photoplethysmograph has been previously reported to accurately record changes in mean arterial pressure (MAP) (15, 16). The finger bearing the photoplethysmograph cuff was positioned at the heart level and kept at the same level for the duration of the study. The electrocardiogram (ECG, Bioamb, ADInstruments, Castle Hill, Australia) was continuously monitored before, during and 20 minutes after infusion of epinephrine. Arterial oxygen saturation (SaO_2) was monitored continuously by pulse oximetry (Poet II, Criticare Systems, Waukesha, USA) with the probe placed on the middle finger of the dominant hand. Analog signals of blood pressure, HR, ECG and arterial oxygen saturation (SaO_2) were continuously recorded and stored on a personal computer (Apple iMac PC) using a PowerLab 16S data acquisition system (ADInstruments, Castle Hill, Australia) at a sampling rate of 100 Hz. From the continuous blood pressure measurement, the arterial pulse wave was analyzed by a pulse wave analysis method, which computes changes in left ventricular stroke volume (SV) from the pulsatile systolic area. The algorithm was the improved method of Wesseling, utilizing the Modelflow program (model-based measurement method based on a nonlinear, 3-element model of the input impedance of the aorta) (10). The measures of SV, derived from the Modelflow value of cardiac output (CO), were calibrated against simultaneous values measured with Doppler ultrasound from the parasternal notch (2 MHz, GE Vivid 3). CO was computed as SV times HR and total peripheral resistance (TPR) was calculated as MAP divided by CO. The assessment of dynamic of changes in the beat-to-beat hemodynamic by using Finometar have become the methods of choice in studies requiring continuous noninvasive recordings. Most validation studies have used intravascular blood pressure measurements in the radial artery as a reference (16, 17).

Ultrasonographic spleen measurements

All ultrasonographic measurements were taken by the same physician, (experienced in abdominal ultrasonography) with a 1.5–3.3 MHz phase array probe (Vivid 3, GE, Milwaukee, WI, USA). The accuracy, realibility, and validity of measuring spleen length and volume by abdominal echograms were reported in an earlier study (18-20). The participants rested in supine position for 30 minutes before baseline measurements were recorded. In this body position the cine loop data were obtained which showed maximal length and maximal width of the spleen in successive time frames lasting 3 s each. Cine loops were acquired through the 10^{th} intercostal space and stored on a hard disk for later analysis. At a later date, three separate measures of the boundaries for length and width of the spleen were identified manually with an electronic caliper by the same author. Repetitive estimates were consistent within 1–2 mm. Cross-sectional area and the estimated volumes of the spleen were calculated as previously described (21). During the infusion of epinephrine, the spleen volume was measured every minute and in 1, 5, 10 and 20 minute after cessation of epinephrine infusion.

Muscle sympathetic nerve activity measurements

To assess the peripheral muscle sympathetic nerve response on continuous epinephrine infusion we measured MSNA, as an index of peripheral sympathetic activation, in conjunction with spleen volume changes and hemodynamic parameters every 30 s. Three of nine subjects were excluded from the analysis due to a poor quality neuronal signal, resulting with six eligible participants.

Microneurography is the only technique that provides direct recordings of sympathetic nerve activity and is commonly used in the studies that assess sympathetic nervous system responses to various stimuli. This method can be used to record muscle (MSNA) or skin (SSNA) sympathetic nerve activity. It is well established that positive correlation exists between the number of bursts in muscle nerves recorded by microneurography and the concentration of the sympathetic transmitter norepinephrine in forearm venous plasma. Furthermore the positive relationship exists between spontaneous MSNA and both spillover of norepinephrine from the heart and concentration of norepinephrine in coronary sinus venous plasma (22-24). Multiunit MSNA of postganglionic sympathetic activity was recorded from the right peroneal nerve with a unipolar tungsten electrode by microneurography (25). The nerve signal was amplified 100,000 times. Afterwards signal was band-pass filtered (0.7–2.0 kHz), rectified and integrated using 0.1 s time constant (662C-4, nerve traffic analysis system, Bioengineering, The University of Iowa, USA). Data were sampled at 1 kHz and stored for subsequent analysis using Chart software (ADInstruments, version 5.5.6.7). MSNA bursts were identified according to following criteria: (1) signal to noise ratio >2; (2) latency limit; (3) burst width limit (short duration = artifact, long duration = skin sympathetic nerve activity or afferent activity; (4) no preceding premature beats. MSNA activity was expressed as frequency of bursts per minute (burst frequency) and per 100 heart beats (burst incidence). Amplitude and area of each burst was calculated. Total MSNA was calculated as the sum of all burst areas per minute.

Data analysis

Results are expressed as the mean ± S.E. Comparisons between parameters before and after epinephrine infusion were

first tested with non-parametric Friedman analysis of variance (because of the small sample sizes; n =13). In case of a significant difference, the Wilcoxon signed-rank test was applied for the particular comparison. All analyses were performed using Statistica 6.0 software (Statsoft, Tulsa, OK, USA).

RESULTS

Spleen volume

The spleen volume decreased 47% (p=0.008) during nine minutes of epinephrine infusion). Most of the splenic volume reduction (around 30%, p=0.008) was accomplished in the second minute (120 seconds) of epinephrine infusion. The recovery phase was slower and it took ~20 minutes for the complete spleen volume recovery (*Fig. 1.*). The maximal reduction in spleen volume was ~50% (*Fig. 1.*) and was observed after nine minutes of epinephrine infusion.

Cardiovascular parameters

Responses in MAP, diastolic pressure, SV, HR, TPR and MSNA are summarized in *Fig. 2*. A significant decrease in TPR (–41%, (p=0.001) was observed 90 s after the onset of epinephrine infusion. The decrease in TPR was counteracted by the parallel increase in cardiac output and the MAP decreased after the onset of epinephrine infusion (–6.2% in the ninetieth second of infusion, p=0.02). The baseline values were restored ~5 min after the cessation of infusion. The decrease in MAP was mainly due to the decrease in diastolic pressure, which significantly decreased 90 s after the onset of epinephrine infusion by ~7.4% (p=0.01). The baseline values were gradually restored during the 10 minutes of recovery. Maximal increase in HR was observed 90 seconds after starting epinephrine infusion, maximally for ~27% (p=0.001). It was normalized 5 min after the end of infusion. SV increased gradually during epinephrine infusion by ~22.5% (p=0.001), (*Fig. 2*).

Sympathetic nervous activity

A subgroup of 9 patients underwent MSNA measurement and in 6 out of 9 subjects good quality recordings were obtained. Average baseline MSNA frequency was 15±1 bursts/min with burst incidence of 27±3 bursts/100 heart beats. After the start of epinephrine infusion MSNA frequency was increased from 15±4 at baseline to 32±7 (120%) in 90th s of continuous epinephrine infusion (p=0.04) (*Fig. 2*). Total MSNA (burst frequency × normalized area) was significantly increased from 505 ± 79 a.u. at baseline to 1116±158 a.u. in ninetieth seconds of the continuous epinephrine infusion (p=0.027).

DISCUSSION

Our results demonstrate that low doses of epinephrine trigger a rapid splenic contraction with concomitant increase in peripheral MSNA. The spleen contracts at the onset of epinephrine infusion, 30 seconds after observed decrease in TPR and MAP and increase in HR, SV and MSNA. These results suggest unloading of baroreceptors and existence of central sympathetic mechanism which initiates early splenic contraction.

The receptor profile of epinephrine is complex, and its pharmacologic effects depend largely on the dose. Low doses of epinephrine (0.1 μg/kg/min) cause a decrease in systemic vascular resistance due to a greater sensitivity of vasodilator β_2-receptors than of constrictor α-receptors (26-28). The effects of catecholamines on spleen are mediated *via* both α- and β-adrenoreceptors, in a way that stimulation of α-adrenoreceptors causes spleen contraction, and stimulation of β-adrenoreceptors causes spleen relaxation (9-11). In our study, low dose epinephrine infusion, which predominately stimulates β-adrenoreceptors, caused significant splenic contraction with 40% reduction of its volume. More than 30% of this reduction was accomplished in the second minute of infusion. This paradoxical and rapid splenic response to stimulation of β-

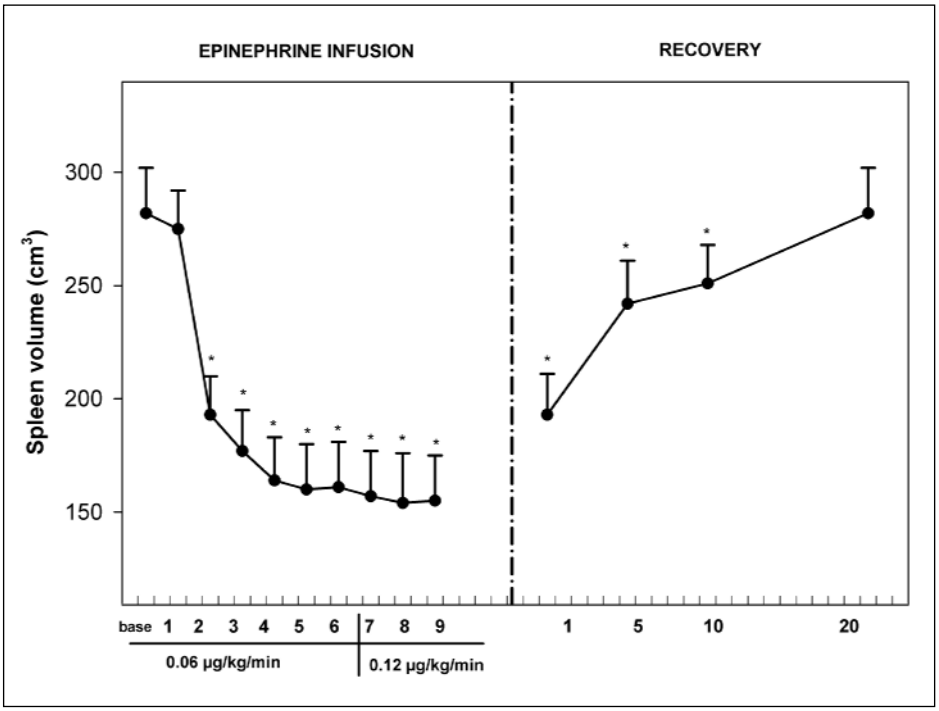

Fig. 1. Ultrasonographically assessed spleen volume, presented as mean ±S.E., before, during, at the end of infusion and in recovery period (1, 5, 10 and 20 min after cessation of epinephrine infusion) in thirteen subjects. *Values are statistically significant (p<0.05) compared to baseline values.

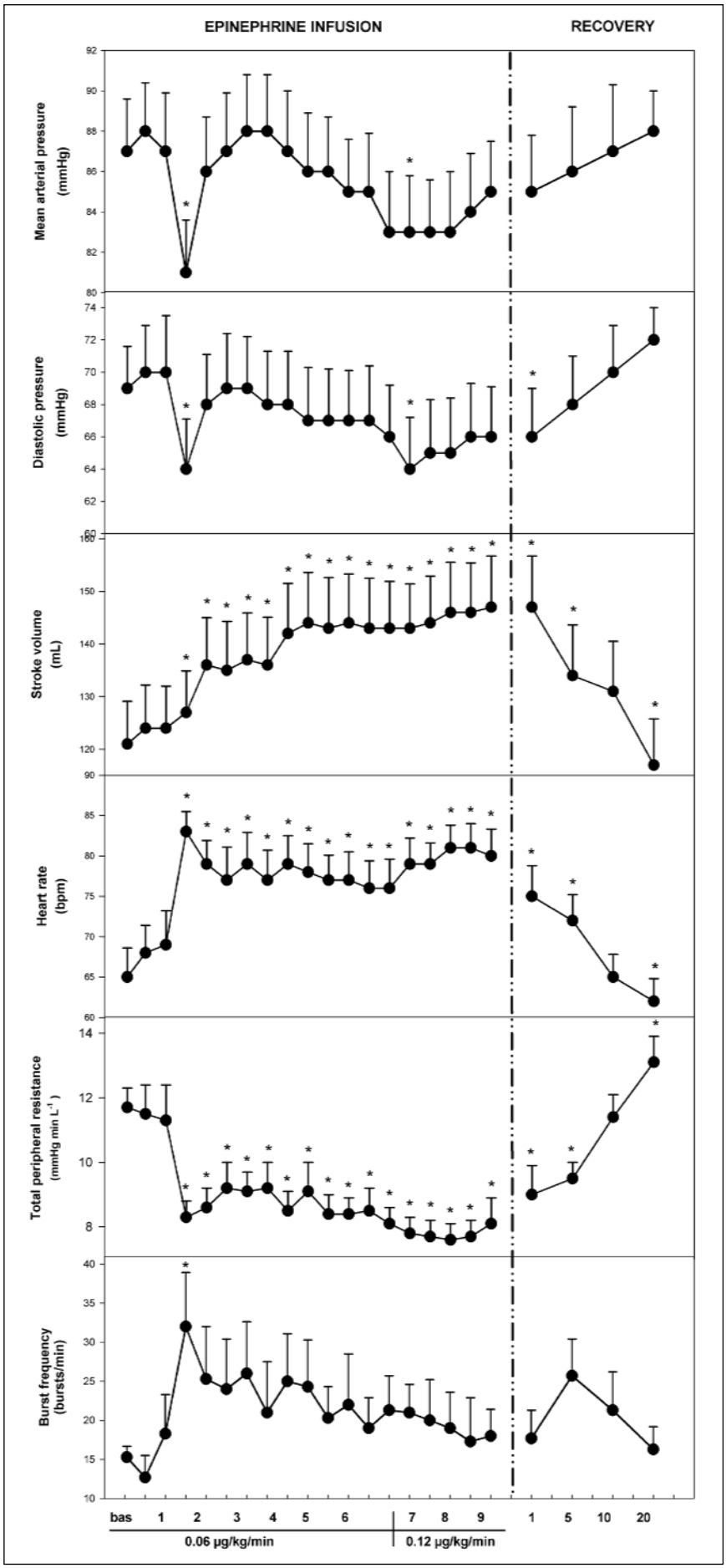

Fig. 2. Responses in mean arterial pressure, diastolic pressure, stroke volume, heart rate, total peripheral resistance in thirteen subjects and muscle sympathetic nerve activity (bursts frequency) in six subjects presented as mean ±S.E. This graph represents values obtained before, every 30 seconds during intravenous epinephrine infusion and in recovery period (1, 5, 10 and 20 min after cessation of epinephrine infusion) in thirteen subjects. *Values are statistically significant ($p<0.05$) compared with baseline values.

adrenoreceptors argues against peripheral triggers and favors the hypothesis of central sympathetic stimulation. The conclusions about the influence of catecholamines on spleen volume were based on the studies in which information about spleen contraction in response to different α- and β-stimulation was reached indirectly by estimation of changes in peripheral platelet count (9-11). We believe that this methodology, without interest in direct spleen volume changes and concomitant cardiovascular fluctuations, masked the genuine physiology of spleen changes in response to different stimuli.

Our assessment of MSNA demonstrates a concordance between an increase in MSNA and the onset of spleen contraction occurring immediately after a decrease in MAP. This finding is consistent with the view that activation of sympathetic fibers in spleen occurs due to unloading of baroreceptors in response to low dose epinephrine which will cause predominantly vascular β_α-adrenoceptor stimulation, thus suggesting involvement of a centrally mediated mechanism in the regulation of spleen response. In our previous studies we have challenged the theory about passive spleen collapse in response to breath hold attempt, which is one of the highest stimulation of sympathetic nerve activity (6, 29). We have previously shown that in simulated apnea diving the reduction of spleen is fast with unchanged flow in splenic artery, which excludes the possibility of passive collapse and shows that in apnea diving the spleen is not the part of periphery with reduced blood flow (12). Palada *et al*. (13) came to similar conclusions that the spleen contraction is present at the very beginning of large lung volume apnea, and probably facilitated by baroreflex inhibition in conditions of decreased blood pressure and cardiac output.

It is well established that exposure to stressors alters activities of the adrenomedullary hormonal system (AHS) and sympathetic nervous system (SNS) with a concomitant increase in AHS and SNS and plasma levels of catecholamines (30). Dive and postdive catecholamine levels collected from freely diving Weddell and harbor seals show a significant increase over resting levels (2, 31). In hooded and harp seals, *in vitro* plethysmographic measurements indicate that α-adrenoreceptor activation with epinephrine results in forceful contraction within 1–3 min of administration. Stimulation of β-receptors and cholinergic receptors did not cause capsular contraction. The contractile effect of epinephrine and nor-epinephrine was largely abolished when the α-adrenergic receptors were first blocked with phentolamine (2, 32) found that, *in vivo*, Weddell seal spleens contract on stimulation by exogenous epinephrine infusion. The splenic dimensions obtained during the postepinephrine injection period were similar to those obtained from the postdive period. However, the doses required to obtain an equal degree of contraction are 400 times resting and significantly higher than observed physiological levels. This finding suggests that direct neural stimulation plays a considerable role in diving-induced splenic contraction (33). Experiments with feline spleens indicated that the capsule is mainly under neural control, demonstrating significant capsular contraction with neural stimulation alone (34). In a diving seal, the rapidity of contraction indicates that the initial stimulation is likely neural in origin, but contraction may be sustained throughout the dive by circulating catecholamines released from the adrenal gland. To further elucidate this topic in humans, measurement of catecholamine plasma concentration in accordance with spleen volume response during different stressors remains to be carried out. For example, Kristek *et al*., shown that prolonged infusion of prazosin can diminish the noradrenaline effect on blood pressure increase, raising the question about its influence on spleen volume (35). Our study is limited by the lack of measurement of the plasma level of epinephrine during continuous infusion. Further research should focus on the responses of spleen contraction to direct α-agonist and antagonist agents, especially when we know its influence on aortic diameter and consequently on blood pressure (36).

The importance of understanding the physiology of spleen originates from its role as reservoir of blood cells. Leukocytes and platelets accumulate in the human spleen accounting for approximately 40% of both body populations (37-39). Spleen contraction induced with apnea resulted in fast and sustained increase in leukocytes in intact persons in comparison with splenectomized subjects, explaining at least in part the stress leukocytosis which occurs in humans in stressful situations (40). Moreover, the human spleen retains one third of body platelets, and mean platelet volume (MPV) of the cells from human spleen is approximately 20% greater than MPV of circulating platelets (41). On the other hand, it has been shown that large platelets are metabolically and enzymatically more active than small platelets and produce more thromboxane A_2 (42-45). Several recent studies have shown a strong connection between high MPV and thrombotic events like acute coronary incidents and stroke (46-48). If we know that these conditions are associated with high level of sympathetic activation than we are coming to conclusion that in these situations the centrally mediated spleen contraction could be an important source of large platelets, thereby increasing the risk of thrombotic incidents (49). In our recent study (50) we found an increase in MPV in response to spleen contraction induced by low dose epinephrine infusion, in conditions of decreased blood pressure. Thus, the spleen is a dynamic reservoir of large platelets, the recognized prothrombotic factors.

In conclusion, we demonstrate that massive spleen contraction occurs at the very beginning of low doses of epinephrine infusion just after the observed decrease in total peripheral resistance and mean arterial pressure and the increase in peripheral sympathetic activation. These findings support the hypothesis that spleen may represent a constitutive part of the sympathetic nervous system during stressful situations.

Acknowledgments: The authors thank Dr. Ana Barac (cardiologist at MedStar Washington Hospital Center, Washington, USA, for help with preparation of the manuscript). This study was supported by the Croatian Ministry of Science, Education and Sports Grant No. 216-2160133-0130.

Conflict of intersts: None declared.

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Received: March 15, 2013
Accepted: September 10, 2013

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