Role of the Multidomain Protein Spinophilin in Blood Pressure and Cardiac Function Regulation

Andrey C. da Costa-Goncalves, Jens Tank, Ralph Plehm, Andre Diedrich, Mihail Todiras, Maik Gollasch, Arnd Heuser, Maren Wellner, Michael Bader, Jens Jordan, Friedrich C. Luft, Volkmar Gross

Abstract—Spinophilin controls intensity/duration of G protein-coupled receptor signaling and thereby influences synaptic activity. We hypothesize that spinophilin affects blood pressure through central mechanisms. We measured blood pressure and heart rate in SPL-deficient (SPL<sup>−/−</sup>), heterozygous SPL-deficient (SPL<sup>+/−</sup>), and wild-type (SPL<sup>+/+</sup>) mice by telemetry combined with fast Fourier transformation. We also assessed peripheral vascular reactivity and performed echocardiography. SPL<sup>−/−</sup> had higher mean arterial pressure than SPL<sup>+/+</sup> and SPL<sup>+/−</sup> (121±2, 112±1, and 113±1 mm Hg). Heart rate was inversely related to spinophilin expression (SPL<sup>−/−</sup> 565±0.4, SPL<sup>+/−</sup> 541±5, SPL<sup>+/+</sup> 525±8 bpm). The blood pressure response to prazosin, trimethapamine, and the heart rate response to metoprolol were stronger in SPL<sup>−/−</sup> than SPL<sup>+/+</sup> mice, whereas heart rate response to atropine was attenuated in SPL<sup>−/−</sup>. Mesenteric artery vasoreactivity after angiotensin II, phenylephrine, and the thromboxane mimetic (U46619) as well as change in heart rate, stroke volume, and cardiac output after dobutamine were similar in SPL<sup>−/−</sup> and SPL<sup>+/−</sup>. Baroreflex sensitivity was attenuated in SPL<sup>−/−</sup> compared with SPL<sup>+/−</sup> and SPL<sup>+/+</sup>, which was confirmed by pharmacological testing. Heart rate variability parameters were attenuated in SPL<sup>−/−</sup> mice. We suggest that an increase in central sympathetic outflow participates in blood pressure and heart rate increases in SPL<sup>−/−</sup> mice. The elevated blood pressure in SPL<sup>−/−</sup> mice was associated with attenuated baroreflex sensitivity and decreased parasympathetic activity. Our study is the first to show a role for the spinophilin gene in blood pressure regulation. (Hypertension. 2008;52:702-707.)

Key Words: autonomic nervous system ■ blood pressure regulation ■ spectral analysis ■ spinophilin-deficient mice ■ telemetry

The intensity and duration of G protein-coupled receptor (GPCR) signaling are regulated by small interacting proteins, including regulators of G protein signaling (RGS) and spinophilin (SPL). RGS2 accelerates guanosine triphosphatase activity, terminates GPCR-dependent signaling, and thereby affects blood pressure (BP) control in the whole animal. SPL also regulates GPCRs, which results in signal attenuation, at least in α-adrenoreceptors (α-AR). Furthermore, SPL recruits RGS proteins to the GPCR complex and thereby increases the efficiency of RGS2 on Ca<sup>2+</sup> signaling blockade after α-AR stimulation. In this way, SPL deletion should have similar effects on BP regulation as RGS2 deletion. However, the mechanisms by which SPL affects BP regulation may be different from those described for RGS2. SPL is especially enriched in neuronal dendritic spines and is involved in the regulation of spine density and synaptic activity, including glutamatergic transmission. Glutamate transmission is believed to play an important role for regulating sympathetic outflow and autonomic control of the cardiovascular system through the nucleus tractus solitarii. We hypothesized that SPL influences BP regulation mainly by modulating central sympathetic/parasympathetic outflow. We measured BP and heart rate (HR) in unrestrained homozygous SPL-deficient (SPL<sup>−/−</sup>), heterozygous SPL-deficient (SPL<sup>+/−</sup>), and wild-type (SPL<sup>+/+</sup>) mice by telemetry combined with fast Fourier transform analysis of mean arterial BP (MAP) and HR to describe spontaneous baroreflex and HR variability (HRV) coupled with pharmacological autonomic testing. We also assessed peripheral vascular reactivity in SPL<sup>−/−</sup> and SPL<sup>+/+</sup> mice and performed echocardiography.
**Methods**

**Animals**

The experiments were performed in adult, 12- to 16-week-old C57Bl/6J29SvJ male SPL-/- mice weighing 24±0.5 g, male SPL+/+ mice weighing 25±1 g, and male SPL+++ mice weighing 25±0.5 g. The generation of SPL-deficient mice was described by Feng et al.11 All animals used in this study were derived from breeder pairs supplied through the courtesy of Dr P. Greengard (Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY) and Dr P. B. Allen (Department of Psychiatry, Yale University School of Medicine, New Haven, Conn) and were bred in our animal facility. The animals were allowed free access to standard chow (0.25% sodium; SNIFF Spezialitäten GmbH, Soest, Germany) and drinking water ad libitum. The local council on animal care approved the study according to requirements of the American Physiological Society. Our approaches to radiotelemetry, spectral analysis, baroreflex sensitivity, pharmacological testing, behavioral stress, vascular reactivity, echocardiography, and biostatistics have been described earlier.3,4,6 Details are available in the online data supplement (see http://hyper.ahajournals.org).

**Results**

**Blood Pressure and Heart Rate in Conscious SPL-/-, SPL+/+, and SPL++/+ Mice**

In Figure 1, day/night MAP (upper panel, left), HR values (lower panel, left), and the averages for the 12 hours day and night MAP (upper panel, right) and HR (lower panel, right) values calculated over 3 days in SPL-/-, SPL+/+, and SPL+++ mice are given. Both parameters showed clear-cut day/night rhythm. SPL-/- mice had increased MAP during the day (119±3 versus 109±3 and 109±2 mm Hg) and night (129±3 versus 119±2 or 118±2 mm Hg), compared with SPL+/+ or SPL++/+ mice. HR was also higher in SPL-/- (day: 542±14, night: 592±17 bpm) compared with SPL+/+ (day: 488±9, night: 527±12 bpm) and SPL++/+ (day: 503±20, night: 550±7 bpm). Whereas BP levels in SPL++/+ and SPL+/+ were similar, HR increased continuously from SPL-/- to SPL++/+ mice.

**Baroreflex Function and Heart Rate Variability**

Baroreflex sensitivity (BRS) calculated by cross-spectral analysis in the low-frequency (LF) band (BRS-LF) or with the sequence method (BRS-up) revealed a stepwise decrease of this parameter from SPL+/+ to SPL-/- (Figure 2, left and middle panels). BRS-up leveled in SPL-/- 1.2±0.1, in SPL+/+ 2.0±0.4, and in SPL++/+ 2.5±0.3 ms/mm Hg. The respective values for BRS-LF were in SPL+/+ 2.2±0.3, in SPL+++ 3.5±0.8, and in SPL++/+ mice 4.4±0.5 ms/mm Hg. LF of HR spectra (LF-HRV) were strongly attenuated in SPL-/- (SPL-/- 29.9±11.6, SPL++/+ 42.1±7.8; Figure 2, right panel). Total power (SPL+/+ 70.0±9.5 ms, SPL++/+ 34.1±5.8 ms2) and root mean square of successive differences (SPL+/+ 7.1±0.7 ms, SPL++/+ 3.6±0.3 ms) were also lower in SPL++/+ mice (Figure S1).

**Pharmacological Testing**

We blockaded peripheral α1-adrenergic receptors with prazosin (Figure 3, upper left panel) and used ganglionic blockade with trimethaphane to investigate the sympathetic drive to the periphery (Figure 3, lower left panel). Prazosin at 0.5 and 1 mg/kg decreased MAP more in SPL-/- (Δ MAP 10±2 and 16±2 mm Hg) than in SPL++/+ mice (Δ MAP 3±1 and 6±3 mm Hg). Trimethaphane at 120 mg/kg decreased MAP in SPL-/- more than in SPL++/+ (Δ MAP 33±3 versus 17±4 mm Hg). Metoprolol at 8 mg/kg (Figure 3, upper right panel) decreased HR stronger in SPL-/- (Δ HR 69±5 bpm) than in SPL++/+ mice (Δ HR 38±9 bpm). The increase in HR after 4 mg/kg atropine (Figure 3, lower right panel) was
smaller in SPL−/− mice (Δ HR 78±5 bpm) than in SPL+/+ mice (Δ HR 119±5 bpm).

Pharmacological Baroreflex
To assess BRS, HR responses to BP increases induced by intravenous bolus injection of phenylephrine (PE) at doses of 2.5, 5, and 10 μg/kg were measured in SPL−/− and SPL+/+ mice. The HR decrease (Δ HR) relative to the prestimulation level in response to the increase in MAP (Δ MAP) was obtained and averaged for each PE dose. The dose-dependent slopes of the baroreflex function (Δ HR/Δ MAP) for SPL−/− and SPL+/+ mice are shown (Figure 4). Whereas at 2.5 μg/kg, PE ΔHR/Δ MAP was not different between SPL−/− (2.7±0.5 ms/mm Hg) and SPL+/+ (3.2±0.8 ms/mm Hg) mice, at 5 and 10 μg/kg PE, the quotient ΔHR/Δ MAP was significantly lower in SPL−/− mice compared with SPL+/+ mice. In SPL−/− mice, ΔHR/Δ MAP leveled at 0.9±1.3 ms/mm Hg (5 μg/kg PE) and 3.9±1.2 ms/mm Hg (10 μg/kg PE). The respective values for SPL+/+ mice were 6.4±2.3 ms/mm Hg and 10.1±3.2 ms/mm Hg.

Behavioral Stress Reaction
MAP increased initially in SPL−/− mice to 138±2 and in SPL+/+ mice to 140±2 mm Hg and declined thereafter in the new environment. In SPL−/− mice, the values decreased during the first 40 minutes to 127±1 mm Hg in SPL−/− and in SPL+/+ mice to 110±1 mm Hg. The BP changes were expressed in percentage of the maximum response for each mouse. The BP decline was slower in SPL−/− than in SPL+/+ mice, as shown by the different slopes (Figure S2, upper panel). The HR increased initially in SPL−/− mice to 683±10 and in SPL−/− mice to 694±5 bpm. The decline, calculated with absolute HR values (SPL−/− 471±10, SPL+/+ mice 560±15 bpm) or as percentage of maximum HR increase, was also different between the groups (Figure S2, lower panel).

Vascular Reactivity
The dose–response curves for angiotensin II (upper panel), for PE (middle panel), and for U46619 (lower panel) were not changed in mesenteric arteries from SPL−/− compared with SPL+/+ mice (Figure S3).

Stress-Echocardiography
Echocardiographic parameters describing left ventricular geometry and the reaction of left ventricular function to sympathic stimulation with dobutamine (stroke volume, HR, and cardiac output) were not different between SPL−/− and SPL+/+ mice (Table S1).
Discussion

The basic observation in our study was that disruption of the *spl* gene in mice increases BP and HR and impairs autonomic regulation after β1-AR blockade, a stronger decrease of BP after ganglionic and α1-AR blockade, and a decrease of BRs. On the other hand, no differences in both the vasoreactivity of isolated mesenteric arteries to vasoconstrictors and in the reaction of left ventricular function to dobutamine stimulation were found. These findings suggest a centrally increased sympathetic tone and an increased peripheral vascular resistance in *spl* gene-deleted mice compared with controls.

Small interacting proteins regulate the intensity and duration of GPCR signaling. SPL modulates at least 2 subfamilies of GPCR, the α2-AR and the D2 dopamine receptor, by blocking G protein receptor kinase and thereby attenuating receptor phosphorylation. Furthermore, SPL regulates GPCR signaling by recruiting RGS proteins and thereby reduces the intensity of Ca²⁺ signaling. In this way, SPL deletion has similar effects as RGS2 deletion. In accord with this view, SPL⁻/⁻ mice displayed approximately 10 mm Hg higher BP during day and night than SPL⁺/⁺ mice. This BP increase was similar to what we observed in RGS2⁻/⁻ mice and was not associated with cardiac hypertrophy, at least in the timeframe of our study. We also found that SPL⁻/⁻ mice had similar BP values compared with SPL⁺/⁺ mice, which suggests that the loss of one *spl* gene copy was compensated.

The most likely explanation for the BP increase in SPL⁻/⁻ mice is an increase in total peripheral resistance, because SPL⁻/⁻ mice showed a stronger BP decrease after α1-AR blockade with prazosin than SPL⁺/⁺ mice. However, the sensitivity of mesenteric vessels to PE and other vasoconstrictors was not different between SPL⁻/⁻ and SPL⁺/⁺ mice. Echocardiographic data at rest and HR, stroke volume, and cardiac output after dobutamine stimulation were not different in SPL⁻/⁻ and SPL⁺/⁺ mice.

We attribute the stronger decrease in BP after α1-AR blockade in SPL⁻/⁻ mice to an increased sympathetic tone rather than to increased sensitivity and/or number of α1-AR in the peripheral vasculature. This view is supported by the stronger effects of ganglionic blockade in SPL⁻/⁻ than SPL⁺/⁺ mice. In this respect, SPL⁻/⁻ mice were different from RGS2⁻/⁻ mice, in which we found an increased vascular sensitivity to vasoconstrictors. Moreover, the BP increase in SPL⁻/⁻ mice was associated with an increase of HR, a combination that is typically found when the sympathetic arm of the autonomic nervous system is activated.

In mice with overexpression of cardiac Gαs, similar to SPL⁻/⁻, an elevated HR and BP was described and discussed as enhanced β-adrenergic signaling. Besides this issue, we found a stronger decrease in HR after β1-AR blockade in SPL⁻/⁻ mice than in SPL⁺/⁺ mice. This sympathetic activation in SPL⁻/⁻ mice may also be responsible for the exaggerated response of SPL⁻/⁻ to environmental stress. MAP and HR declined more slowly in SPL⁻/⁻ than in SPL⁺/⁺ when the mice were placed in a new environment.

To provide further insight into autonomic control of the cardiovascular system in SPL⁻/⁻ mice, we used HRV and determined spontaneous BRS. Spontaneous BRS, calculated with the sequence technique and with spectral function analysis of spontaneous changes in systolic blood pressure and HR, was decreased in SPL⁻/⁻ mice. Blunted baroreceptor reflex HR control has been described as a characteristic response in hypertension with high sympathetic tone, a combination we observed in the SPL⁻/⁻ mice. The change in HR regulation was also reflected in the LF-HRV data. In humans, LF of HRV is predominantly influenced by the sympathetic tone, whereas the high-frequency component of HRV is mainly vagally controlled. On the contrary, in mice, high-frequency oscillations are at least a part of mechanical origins, whereas LF oscillations of HRV are mainly under parasympathetic control. Because LF-HRV was decreased in SPL⁻/⁻ mice, we suggest a decreased parasympathetic activity in these mice. Furthermore, SPL⁻/⁻ showed a reduction in root mean square of successive differences and a smaller BP decrease after atropine than SPL⁺/⁺ mice, which underlines this assumption. Experimental and clinical evidence has been provided that an increase in sympathetic activity contributes to a decrease in BRS. Therefore, the attenuated BRS in SPL⁻/⁻ mice could be caused by an increase in sympathetic tone and/or a decrease in parasympathetic tone in these mice.

The spontaneous baroreflex describes the BRS at the operating point of the BP and may differ from the maximum baroreflex gain. To validate spontaneous BRS as a valid parameter describing the baroreflex in mice, we measured changes in MAP and HR to increasing concentrations of PE. The baroreflex gain of this pharmacological baroreflex was also attenuated in SPL⁻/⁻ mice. To our knowledge, this is the first study that compared both methods in mice. This result underlines the validity of spontaneous BP and HR changes to characterize BRS in conscious mice as previously shown in humans.

The mechanism of increased central sympathetic tone in SPL⁻/⁻ mice is an open question. SPL attenuates signaling of α2-AR, including the α2A-AR, which are mainly responsible for central sympathetic outflow. Deletion of the *spl* gene should result in prolongation of α2-AR

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**Figure 4.** Pharmacological baroreflex determined as the ratio of heart rate change over mean arterial pressure change (ΔHR/ΔMAP) after PE in SPL⁺/⁺ and SPL⁻/⁻ mice. Baroreflex gain was reduced in SPL⁻/⁻ mice. *P<0.05.
activation with the result of a decrease in sympathetic drive and a reduction in BP and HR. This view is not supported by our results.

Stimulation of N-methyl-d-aspartic acid receptors within the paraventricular nucleus increases sympathetic tone, which is associated with an increase of BP and HR. Activation of \(\alpha_1\)-ARs or \(\alpha_2\)-ARs inhibits N-methyl-d-aspartic acid receptor currents. SPL selectively facilitates RGS2/4 modulation of \(\alpha_2\)-AR effects on N-methyl-d-aspartic acid receptor currents. We speculate that deletion of the spl gene attenuates the RGS2/4 effect and decrease \(\alpha_1\)-ARs dependent inhibition of N-methyl-d-aspartic acid receptors. As a consequence, centrally originated sympathetic tone could increase. Another binding partner of SPL is protein phosphatase-1, which modulates the activity of a variety of ion channels, thereby affecting receptors, including the glutamate receptor. Glutamate receptors are involved in cardiovascular reflexes and play a role in regulating sympathetic tone and cardiovascular function.

Our data suggest that an increase in the sympathetic peripheral outflow to the resistance vessels plays a role for increase of BP and HR in SPL-deficient mice. The elevated BP in SPL-deficient mice was associated with an attenuated baroreceptor reflex and compromised parasympathetic activity. Despite the pathways and the cellular mechanisms into how SPL affects BP regulation are not defined, our study is the first to show that the spl gene is involved in BP regulation. Taking into consideration that an increased sympathetic outflow plays an important role in hypertension and that stimulation of cardiac nerves is thought to be a powerful predictor of death in heart failure, a reduced expression of SPL may contribute to the changes in BP regulation through the autonomic nervous system and thereby contribute to an increase of BP.

Perspectives

Our data serve to add spl to the list of genes important for BP regulation and probably the development of hypertension. The cardiovascular function of the human homolog should be pursued.

Acknowledgments

We thank Ilona Kamer (Max Delbrück Center for Molecular Medicine, Berlin, Germany) and Diana Herold (Charite University Medicine, Section Nephrology/Intensive Care and Franz Volhard Clinic at the MDC) for technical assistance.

Source of Funding

The Deutsche Forschungsgemeinschaft supported this study (Gr 1112/13-1: Go 766/5-5).

Disclosures

None.

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Hypertension. 2008;52:702-707; originally published online August 18, 2008; doi: 10.1161/HYPERTENSIONAHA.108.114355
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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The role of the multi-domain protein spinophilin in blood pressure and cardiac function regulation.

Short title: Spinophilin regulates cardiovascular function

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Method

All protocols were approved by the local council on animal care and correspond to requirements of the American Physiological Society.

Telemetry

We measured blood pressure (BP) and heart rate (HR) in SPL -/- (n=8), SPL +/- (n=5) and SPL +/+ (n=8) mice by telemetry combined with fast Fourier transform analysis of BP and HR. TA11PA-C20 BP devices (Data Sciences International, St. Paul, MN, USA) were implanted in mice aged between 12-16 weeks to follow BP and HR changes continuously over time. The telemetric techniques and the techniques employed to analyze the autonomic nervous system are described in detail elsewhere. Briefly, we anesthetized mice by isoflurane (CuraMed Pharma GmbH, Karlsruhe, Germany). Then, we advanced the pressure-sensing catheter of the TA11PA-C20 device through the right femoral artery into the abdominal aorta and placed the transmitter in a subcutaneous pocket along the right flank. Mice recovered for 7 days before baseline BP and HR values were recorded. By this time, the mice had regained their circadian BP and HR rhythm; and surgery and anesthesia-induced changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and HR had abated.

Data were sampled every 5 min for 10 sec continuously day and night and stored on a hard disk. SBP, DBP, and HR were recorded using the DATAQUEST software (A.R.T. 2.1, Data Sciences International). Continuous beat-by-beat values of BP and HR were recorded during morning hours for spectral analysis and pharmacological testing. HR was computed from the pulse intervals of the BP recordings. Measurements to calculate the baroreceptor heart rate reflex, HR variability (HRV), and to analyze the autonomic control of BP and HR were performed between 8:00 and 11:00 AM.

Spectral Analysis:
For evaluation of cardiovascular function, the baroreceptor heart rate reflex and HRV in the low frequency range (HRV-LF) were investigated using spontaneous changes in BP and HR. The power spectra of SBP, pulse interval time series, and the cross spectra were calculated using fast Fourier transformation (FFT). Low-frequency components of pulse intervals spectrum (LF), root mean square of successive differences between adjacent normal pulse intervals (RMSSD), total power and the baroreflex sensitivity (BRS-LF) were calculated.

Beat-to-beat values of detected R-R intervals and BP values were interpolated, low-pass filtered (cutoff 6 Hz) and re-sampled at 12 Hz. Data segments of 43 seconds were used for spectral analysis. Linear trends were removed and power spectral density was estimated with the FFT-based Welch algorithm using segments of 512 data points with 50% overlapping and Hanning window. The power in the frequency range of low frequencies (LF: 0.25 to 0.6 Hz) was calculated. Five representative intervals were chosen for spectral analysis and averaged according to the following criteria: 1) steady state conditions, 2) no large sudden BP changes, 3) no artifacts. The frequency bands were adapted for analysis in mice considering the ranges of HR and breathing frequencies.

**Baroreceptor heart rate reflex**

The baroreceptor heart rate reflex was investigated using sequence technique\(^2,3\) and spectral function analysis of spontaneous changes in SBP and HR.\(^1,4\)

**Sequence Technique:** Spontaneous baroreflex slope was calculated as the slope of the linear regression line between SBP and the subsequent R-R intervals using sequences defined as an episode of at least three heart beats with more than 0.01 mmHg SBP per beat. The averaged values of all slopes with a correlation coefficient greater than 0.85 were calculated for sequences with rising BP (BRS-up).

**Baroreflex sensitivity (BRS)** was defined as the mean magnitude value of transfer function between SBP and R-R interval in the low-frequency band (BRS-LF) with negative phase and
squared coherence value greater than 0.5. A negative phase indicates that BP leads R-R-interval and a coherence value greater 0.5 indicates a dominating linear relationship. The data analysis was performed with the PV-wave software (Visual Numerics, Houston, TX, U.S.A.).

**Pharmacological testing**

To evaluate autonomic control of BP the following drugs were applied: muscarinic blockade was obtained with atropine (2 mg/kg, SPL +/+ n=12; SPL +/- n=14), β₁-adrenergic receptor blockade with metoprolol (4 mg/kg, SPL +/+ n=7; SPL +/- n=8), α₁-adrenergic receptor blockade with prazosin (0.5 mg/kg and 1 mg/kg, SPL +/+ n=11; SPL +/- n=11), ganglionic blockade with trimethaphane (120 mg/kg, SPL +/+ n=7; SPL +/- n=6). All substances were given intraperitoneally (IP) in the morning hours between 9:00 AM and 11:00 AM. Continuous beat-by-beat values of BP and HR were recorded for 1 h, after which the mice were briefly removed and drugs were applied. Thereafter beat-by-beat values were recorded for 1 additional hour. As in our former study the values (45th to 60th min) after drug injection were used to characterize the respective responses in order to avoid the measurement of stress-induced BP and HR changes. The protocols for the single injections were separated by at least 24 h. We did not randomize the order of drug administration.

The pharmacological baroreflex was evaluated in SPL +/- (n=9) and in SPL +/+ (n=5) mice. The mice were anesthetized by intraperitoneal (IP) injection of ketamine (100 mg/kg) and xylazine (10 mg/kg), and catheters were placed into the abdominal aorta via the femoral artery for the measurement of arterial pressure and in the femoral vein for drug application. The catheters were tunneled subcutaneously, exteriorized, and sutured between the scapulae. After a recovery period of 48 to 72 hours the baroreflex was tested by intravenous injection of phenylephrine (PE, 2.5, 5.0 and 10.0 µg/kg). PE was given as a bolus (1 µl per 10 g of body weight) when the mice were at rest and BP and HR values showed stable steady state values. BP and HR were recorded with a transducer (MLT 1050 model), connected to a computer system for data acquisition and analysis (PowerLab, ADInstruments) in freely moving mice.
The maximal HR responses relative to the HR baseline levels ($\Delta$ HR) and the maximal MAP response relative to the MAP baseline levels ($\Delta$ MAP) for each PE dose were calculated. The ratio of $\Delta$ HR over $\Delta$ MAP was then calculated and averaged at each dose for each drug in each animal group. The averaged ratio of HR change over MAP change ($\Delta$HR/$\Delta$MAP) was used as an estimate for BRS.

**Behavioral stress**

Mice were exposed to a new environment to induce behavioral stress (SPL -/- n=10, SPL +/- n=9). The mice were removed from their home cages and placed into an unfamiliar cage for 40 min. BP and HR were recorded continuously while the mice explored their new environment. BP and HR changes were expressed as response in % of the maximum response and thereafter analyzed using linear regressions.

**Vascular reactivity**

Intact 2nd or 3rd order branches of mesenteric arteries were obtained from SPL -/- (n=5) and SPL +/- (n=5) mice and quickly transferred to cold (4°C) oxygenated (95% O2/5% CO2) physiological salt solution (PSS) of the following composition (in mM): 119 NaCl, 4.7 KCl; 25 NaHCO3, 1.2 KH2PO4; 2.5 CaCl2, 1.2 MgSO4; and 11 glucose.5 Thereafter, we carefully removed the connective tissue with scissors. The arteries then were mounted onto two glass cannulas in an arteriograph with continuous superfusion (3 to 5 mL/min) of oxygenated PSS at 37°C. One cannula was connected to a reservoir, which produced the desired intravascular pressure by a pressure control system (PS/200, Living Systems Instrumentation, Burlington, Vermont, USA); the other cannula was connected to a pressure monitor (PS/200, Living Systems Instrumentation, Burlington, Vermont, USA). Leaking vessels were discarded at any stage of the experiment to ensure complete non-flow conditions. After a 20-minute equilibration period, intravascular pressure was increased gradually from 2 mm Hg to 60 mm Hg. After an additional period of 30 minutes and before starting the experiment, the vessel was constricted with KCl 60 mmol/L to assess its contractility. Subsequently, KCl was
removed and dose-response curves for angiotensin II (Ang II), PE and the thromboxane mimetic (U46619) were performed. The vessel diameter was measured using a video-microscopic system (Nikon Diaphot, Duesseldorf, Germany), connected to a computer with appropriate software (TSE, Bad Homburg, Germany) for detection of changes of vessel diameter. All vessels tested were from different animals. All drugs were applied to the bath solution, namely from the extraluminal side of the vessel.

**Stress-echocardiography**

Mice (SPL +/+ n=10; SPL -/- n=6) were anesthetized with 2% isoflurane and kept warm on a heated platform. Temperature and ECG were continuously monitored. Cardiac function and morphology were assessed by echocardiography with a VisualSonics Vevo 770 High-Resolution Imaging System with the use of a high-resolution (40 MHz) transducer. Stroke volume (SV) and cardiac output (CO) was measured by tracing the endocardium in systole and diastole of a parasternal long axis view of left ventricle. Stress-shortening relation measurements were performed at baseline and after injection of the β-agonist dobutamine (4 µg/g BW IP).

**Statistics**

Data is presented as mean±SEM. Statistically significant differences in mean values were evaluated by ANOVA followed by Bonferroni post hoc test. For paired data we used the nonparametric Wilcoxon signed rank test. Linear regression analyses were used to describe MAP and HR changes after environmental stress. Slopes and intercepts of data sets were tested for significance using software from GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA). The method is equivalent to an analysis of covariance (ANOVA). P values <0.05 was used to determine statistical significance.
Supplement references


Table S1. Echocardiographic analysis of wild type and SPL-deficient mice

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<tr>
<td>IVSs (mm)</td>
<td>0.88±0.1 [0.98]</td>
<td>1.05±0.05 [1.07]</td>
</tr>
<tr>
<td>LVIDs (mm)</td>
<td>3.2±0.1 [3.2]</td>
<td>3.2±0.1 [3.2]</td>
</tr>
<tr>
<td>FS (%)</td>
<td>25.2±1.5 [26.1]</td>
<td>24.4±1.6 [25.0]</td>
</tr>
<tr>
<td>LV mass/BW (%)</td>
<td>4.2±0.1 [4.2]</td>
<td>4.5±0.2 [4.4]</td>
</tr>
</tbody>
</table>

Cardiac dimensions: LV, left ventricle; LVPWd, LV posterior wall, diastole; IVSd, interventricular septum, diastole; LVIDd, LV internal dimension, diastole; LVPWs, LV posterior wall, systole; IVSs, interventricular septum, systole; LVIDs, LV internal dimension, systole; FS, fractional shortening; BW, body weight; SV, stroke volume; CO, cardiac output. Data shown are mean±SEM and [median]. *p<0.05 before vs. after dobutamine (4.0 μg/g BW).
S 1. Total power (upper panel) and root mean square of successive differences (RMSSD, lower panel) in SPL -/- and SPL +/+ mice. Total power and RMSSD were reduced in SPL -/- mice. (* p<0.05)
S 2. Regression lines for mean arterial blood pressure (MAP, upper panel) and heart rate (HR, lower panel) decline in SPL -/- and SPL +/+ mice exposed to a new environment. The difference between the slopes calculated on the basis of % of the maximum MAP and HR responses were highly significant (p= 0.004). In terms of MAP and HR changes, SPL +/+ mice adapted faster to the new environment than SPL -/- mice.
S 3. Constriction of mesenteric arterioles induced by angiotensin II (Ang II, upper panel), phenylephrine (PE, middle panel), and the tromboxane mimetic U46619 (lower panel) in SPL -/- and SPL -/- mice. The vasoconstrictor responses to Ang II, PE, and U46619 were not different between SPL +/- and SPL -/- mice.