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Dietary soy modulates endothelium-dependent relaxation in aged male rats: Increased agonist-induced endothelium-derived hyperpolarising factor and basal nitric oxide activity

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Abstract

We examined the effects of dietary soy on the contributions of endothelium-derived hyperpolarising factor (EDHF), nitric oxide (NO), and oxidative stress to vascular tone in isolated aortic rings and small mesenteric and pulmonary arteries in vitro. Male Wistar rats were either continuously fed a soy-deficient diet (SD) or switched from a soy-deficient diet to a soy-rich one for 6 months (SW). Contractile responses were generally smaller in arteries from SW rats. In mesenteric arteries, this difference was blunted by L-NAME, but not by charybdotoxin and apamin. Precontracted SW mesenteric arteries were more sensitive to acetylcholine (ACh) than SD ones. This difference was unaffected by L-NAME but was abolished by charybdotoxin and apamin. Exogenous superoxide dismutase (SOD) and catalase induced powerful relaxations in aortic rings, which were smaller in those from SW rats. In mesenteric and pulmonary arteries, however, they partially inhibited ACh-mediated relaxation, and enhanced PGF_{2α}-mediated contraction, respectively. Our results suggest that feeding aging male rats a soy-rich diet results in improved agonist-mediated EDHF production and a generalized reduction in contractile force, which is partly due to elevated basal NO. Our data also suggest a prorelaxant role for endogenous H₂O₂ in small arteries, which is modulated by a soy diet.

Keywords

Dietary soy; Endothelium; Smooth muscle; EDHF; Nitric oxide; Oxygen radicals

Introduction

There is an ongoing debate as to whether soy dietary supplements are of therapeutic use against a variety of cardiovascular diseases in humans [1–3]. In a number of rat models, however, improvements in several cardiovascular risk factors are striking [4–7]. Such

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benefits may be due to the presence in soy of the natural isoflavone phytoestrogens genistein and daidzein which mimic the known cardioprotective actions of endogenous estrogens in premenopausal women [8], or of some other properties of soy protein [7]. Importantly, most standard rat and mouse chows are derived from soy products and are used without consideration by researchers of their effects on the animals' physiology and responsiveness of arterial preparations in vitro.

Recently, we have shown that long-term feeding of a soy-rich diet during gestation and adult life resulted in decreased oxidative stress, improved aortic and pulmonary artery endothelial function, and reduced blood pressure in vivo in aging male rats, compared to littermates fed a soy-deficient diet [4]. We also found that the soy-rich diet increased expression of eNOS and the antioxidant enzymes manganese superoxide dismutase and cytochrome *c* oxidase and that elevations in blood pressure, impaired endothelial function, and reduced eNOS and antioxidant gene expression in animals fed a soy-deficient diet were all reversed after refeeding a soy-rich diet for 6 months [4].

Endothelium-dependent relaxation in aorta and large proximal arteries is dependent almost entirely on NO, and is very sensitive to levels of endogenous superoxide [9,10]. In distal small arteries, however, including those of the mesenteric circulation and to some extent the pulmonary circulation, endothelium-derived hyperpolarising factor (EDHF) is of increasingly greater significance in agonist mediated relaxation as the diameter of the artery decreases [11,12]. EDHF is implicated in control of blood pressure and regional blood flow in mammalian tissues in vivo [13], and may be altered under pathophysiological conditions such as hypertension and diabetes [14,15].

In the present study we have therefore examined the influence of EDHF and NO on vascular tone in small mesenteric arteries of aged male rats fed a soy-deficient or a soy-rich diet. To test for the effects of diet-induced changes in endogenous reactive oxygen species, we examined the acute effects of antioxidant enzymes in aorta and mesenteric and pulmonary arteries. The effects of a soy diet on several contractile responses were also compared, including hypoxic pulmonary vasoconstriction (HPV), a phenomenon believed to be largely endothelium dependent [16].

Methods

Animals

Adult male Wistar rats (up to 20 rats from each group; weighing between 650 and 1000 g at time of sacrifice; kind gift of Prof. Richard Sharpe, MRC Human Reproductive Sciences Unit, Edinburgh, UK) were used for this investigation. Our maintenance of animals conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). All rats were nourished in utero, and fed a standard SDS soy-deficient (SD) diet up to the age of 10 months post weaning [17]. At the age of 10 months they were divided into two groups and either: (1) continued on SD diet for a further 6 months prior to sacrifice or (2) switched from SD diet to a diet rich in soy protein containing the isoflavones genistein and daidzein (SW), for a further 6 months prior to sacrifice. Genistein levels in soy-deficient and soy-rich diets

were 3.44 and 202 mg kg^{-1} , respectively, and in the plasma of rats fed these diets were 2.4 ± 2 and $126 \pm 33 \text{ ng ml}^{-1}$, respectively. Rats were killed by cervical dislocation as prescribed by the UK Home Office. All rats used in the present study were from the same colony as those used in our earlier study, in which SD rats were shown to be hypertensive and oxidatively stressed [4].

Isolated arterial preparations

Segments of thoracic aorta of $\sim 3 \text{ mm}$ diameter were mounted vertically onto isometric force transducers in an organ bath chamber. A 1-g (9.81 mN) resting tension was applied, and, following a 1-h equilibration period, rings were primed with one 5-min exposure to 80 mM K^+ (physiological salt solution; isotonic replacement of NaCl by KCl) and two exposures to phenylephrine ($1 \mu\text{M}$). Third-order mesenteric arteries ($250\text{--}300 \mu\text{m}$ internal diameter) and second- or third-order intrapulmonary arteries ($450\text{--}500 \mu\text{m}$ internal diameter) were dissected from the small intestine and lung of these rats, respectively. These were mounted on an automated Mulvany-Halpern wire myograph (DMT A/S, Aarhus, Denmark), bathed in PSS (gassed with $95\% \text{ air}/5\% \text{ CO}_2$, $\text{pH } 7.4$ at 37°C), and stretched to 90% of the circumference necessary to create an equivalent transmural pressure of 13.4 kPa (100 mm Hg , mesenteric only) or 4.4 kPa (30 mm Hg , pulmonary only). Mesenteric arteries were equilibrated with two 3-min contractions to K^+PSS and one 3-min contraction to $10 \mu\text{M}$ phenylephrine, and pulmonary arteries were equilibrated with three 3-min contractions to $80 \text{ mM K}^+\text{PSS}$ prior to beginning experiments [18]. Hypoxic pulmonary vasoconstriction was induced in pulmonary arteries by gassing with $1\% \text{ O}_2$ ($5\% \text{ CO}_2$, balance N_2) following precontraction with a low concentration of prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$) sufficient to cause a contraction $10\text{--}15\%$ of that induced by K^+PSS [18].

Perfused mesenteric arterial bed

Mesenteries from both 12-month-old and 16-month-old rats were used. A laparotomy was performed and the intestines were deflected. The main mesenteric artery was then cleaned of fatty and connective tissue and cannulated via a small cut. The cannula was then tied with sutures and the mesentery surgically isolated and mounted in an organ bath and perfused with PSS (see below, 6 ml/min , gassed with $95\% \text{ O}_2/5\% \text{ CO}_2$ at 37°C). Perfusion pressure was measured and recorded via an A/D Instruments interface and PowerLab software. After a 20-min stabilization period, the perfusion pressure was set to zero prior to the application of drugs. Drugs were applied as bolus injections, so that, due to dilution within the vascular bed, all concentrations therefore refer to the bolus concentrations, not actual concentrations at the site of action.

Solutions, drugs, and chemicals

Physiological salt solution (in mM): $\text{NaCl } 118$; NaHCO_3 24 ; $\text{KCl } 4$; CaCl_2 1.8 ; MgSO_4 1 ; NaH_2PO_4 0.434 , glucose 5.56 . Acetylcholine, phenylephrine, N^ω -nitro-L-arginine methyl ester (L-NAME), superoxide dismutase, and catalase were purchased from Sigma (Poole, UK), charybdotoxin and apamin from Bachem (St. Helens, UK), and $\text{PGF}_{2\alpha}$ from Biomol (PA, USA).

Statistics and curve fitting

Relaxation concentration response curves were fitted by nonlinear regression and EC₅₀ values calculated using SigmaPlot (SPSS Inc.) All data were expressed as mean and standard error (SE) and (unless otherwise stated) compared by Student's *t* test (paired or unpaired where appropriate), or two-way ANOVA (for multiple comparisons) using SigmaStat (SPSS Inc.). *p* < 0.05 was taken as an indication of significant differences between means.

Results

Responses to contractile agonists

There was no significant difference in developed force between preparations from SD and SW rats when aortic rings were contracted with 1 μ M phenylephrine for 15 min (SD, 11.4 $\pm$ 1.7 mN, *n* = 20; SW, 9.7 $\pm$ 1.0 mN, *n* = 20). Mesenteric arteries were exposed to K⁺PSS for 3 min and then after washing and recovery, to 10 μ M phenylephrine for 7 min (sufficient time for contraction to peak). As shown in Fig. 1A, both K⁺PSS and phenylephrine-induced contractions were significantly smaller in these arteries from SW rats compared to SD rats. Pulmonary arteries were exposed first to K⁺PSS for 3 min and then after washing and recovery, to 30 μ M PGF_{2 α} for 7 min. Again, both K⁺PSS- and PGF_{2 α} -induced contractions were significantly smaller in arteries from SW rats compared to SD rats (Fig. 1B).

Examination of tonic NO and EDHF during phenylephrine contraction

Inhibition of NO synthase with L-NAME (100 μ M) enhanced phenylephrine-induced contraction in aortic rings to the same extent for SD and SW groups (SD, 40.9 $\pm$ 4.4%, *n* = 20; SW, 37.2 $\pm$ 5.1%, *n* = 20). In contrast, L-NAME enhanced phenylephrine-induced contraction in wire myograph-mounted mesenteric arteries of SW rats nearly threefold more than for those from SD rats. However, the modest additional enhancement in contraction elicited by subsequent application of 100 nM charybdotoxin and 100 nM apamin (in the continued presence of L-NAME) was not different between the two groups (For example traces, see Fig. 2A; for mean contraction amplitudes, see Fig. 2B; and for % changes in amplitudes induced by inhibitors, see Fig. 2C). Charybdotoxin and apamin block the intermediate conductance and small conductance Ca²⁺-activated K⁺ currents, respectively, both found exclusively on the endothelium and believed to be necessary for the release and/or action of EDHF [19,20]. After combined blockade of both eNOS and EDHF, phenylephrine contraction amplitude remained smaller in SW rats (Fig. 2B). These results suggest that basal release of NO, but not EDHF in mesenteric arteries, is influenced by a soy diet and has a much greater influence on phenylephrine-mediated contraction in SW rats.

Responses to acetylcholine in mesenteric arteries

Wire myograph-mounted mesenteric arteries were preconstricted with 10 μ M phenylephrine and, after these contractions had stabilised, they were exposed to increasing concentrations of acetylcholine (ACh). Arteries from SW rats were modestly but significantly more sensitive to ACh than those from SD rats (EC₅₀: SD 31.5 $\pm$ 5.2 nM, *n* = 19 vs SW 20.8 $\pm$ 3.5 nM, *n* = 20, *p* < 0.05), but there was no difference in maximum relaxation. In order to

determine whether the NO or the EDHF components of this relaxation were differentially affected by the change of diet, ACh concentration responses were repeated in the presence of the eNOS inhibitor L-NAME (100 μ M), and then, in the continued presence of L-NAME, charybdotoxin and apamin (both 100 nM). L-NAME caused both a rightward shift in the sensitivity (EC_{50}) to ACh and a reduction in the maximum relaxation response (Fig. 3A). EC_{50} values in the presence of L-NAME were SD 0.29 ± 0.07 μ M, $n = 11$ vs SW 0.14 ± 0.04 μ M, $n = 10$ ($p < 0.05$). Compared to control responses, this L-NAME-induced rightward shift in EC_{50} ($-\log M$) was greater in SD (0.93 ± 0.17) than in SW (0.64 ± 0.22), suggesting that L-NAME exaggerated rather than diminished the difference in ACh sensitivity between the two groups. This result suggests that the greater overall sensitivity to ACh in SW versus SD was not due to improved NO production. In both groups of rats, all remaining ACh-induced relaxation was abolished after further addition of charybdotoxin and apamin (in the continued presence of L-NAME, Fig. 3A, triangles), suggesting that the differences between arteries from SW and SD rats resided mainly in the EDHF component of ACh-mediated relaxation. To confirm that the L-NAME-induced rightward shifts in ACh sensitivity were not due to desensitisation of the arteries upon repeated exposures to ACh, a separate series of three control ACh concentration responses was performed in arteries from another group of soy-fed rats ($n = 3$). No differences in either EC_{50} or maximum relaxation were detected (data not shown).

In order to examine endothelium-dependent relaxation in the presence of flow, we repeated the above comparison in perfused mesenteric arterial beds. Following precontraction with continuous perfusion of 10 μ M phenylephrine increasing bolus concentrations of ACh were applied. ACh reduced perfusion pressure in a concentration-dependent manner (Fig. 3B), and preparations from SW rats were significantly more sensitive to ACh than those from SD rats (EC_{50} : SD, 0.4 ± 0.2 μ M, $n = 6$ vs SW, 0.05 ± 0.02 μ M, $n = 5$, $p < 0.05$), whereas maximum relaxation was unaffected.

Effects of superoxide dismutase (SOD) and catalase (cat) on contraction and relaxation responses

To evaluate the influence of oxidative stress on arterial contractile responses, we examined the combined effects of acute treatment with the antioxidant enzymes superoxide dismutase (100 U/ml) and catalase (100 U/ml) on phenylephrine-mediated contractions in aorta and mesenteric arteries and on $PGF_{2\alpha}$ -mediated contraction in pulmonary arteries. In aortic rings, SOD/cat (applied for 20 min after 15 min precontraction to 1 μ M phenylephrine) caused large sustained relaxations and these were significantly smaller in SW compared to SD (Figs. 4A and 4B). In mesenteric arteries, however, SOD/cat (applied for 15 min after 2 min exposure to 10 μ M phenylephrine) did not cause relaxation in either group (Fig. 4B). We also repeated ACh concentration responses in the presence of SOD/catalase. As shown in Fig. 4C, SOD/cat significantly shifted the concentration response to the right in both groups of arteries (EC_{50} : SD, 0.11 ± 0.02 μ M, $n = 8$; SW, 0.10 ± 0.04 μ M, $n = 10$; $p < 0.05$ vs control for both groups). However, under these conditions there was no longer a significant difference in EC_{50} between SD and SW. Additional experiments (in a different cohort of soy-fed male rats, age <6 months) showed that this effect of SOD/catalase on ACh-mediated relaxation persists in the presence of L-NAME, indicating that it is inhibition of

the EDHF component of the relaxation that underlies the rightward shift (EC_{50} : L-NAME, $0.022 \pm 0.005 \mu\text{M}$; L-NAME +SOD/catalase, $0.123 \pm 0.05 \mu\text{M}$, $n = 6$; $p < 0.01$ by paired t test). In these experiments there was no change in the maximum relaxation to ACh.

In $\text{PGF}_{2\alpha}$ -precontracted pulmonary arteries, SOD/cat (applied for 10 min after a 6-min exposure to $\text{PGF}_{2\alpha}$) caused biphasic responses comprising rapid transient relaxations followed by slower sustained constrictions (e.g., Fig. 5A). After the application of SOD/cat, contraction amplitude still tended to be smaller in pulmonary arteries from SW rats, but this difference was no longer significant (Fig. 5B). This was due to a proportionally greater SOD/cat-induced sustained increase in amplitude in the SW arteries (Fig. 5C).

Hypoxic pulmonary vasoconstriction

Pulmonary arteries from both SD and SW rats produced biphasic contractile responses to hypoxia similar to those described in earlier reports using younger rats [18,21,22], comprising a rapid transient first phase and a sustained slowly developing second phase, which relaxed back toward the prehypoxic level upon reoxygenation (e.g., Fig. 6A). There was no difference in the size of the first-phase contraction between groups; however, the second-phase contraction was significantly smaller in arteries from SW rats compared to SD rats ($p < 0.01$ by two-way ANOVA) (Fig. 6B).

Discussion

NO and EDHF in aorta and mesenteric artery

Vascular resistance, a major determinant of blood pressure in vivo, is regulated in small arteries and arterioles of the systemic circulation largely through changes in the release or action of endothelium-derived vasoactive substances. As a mediator of endothelium-dependent relaxation, EDHF is an important determinant of vascular resistance, blood pressure, and regional blood flow [13,23]. We therefore examined the influence of switching rats raised on a soy-deficient diet to one rich in soy on contractile responses and both basal and ACh-mediated NO and EDHF activity in rat resistance mesenteric arteries.

We found that K^+PSS - and phenylephrine-mediated contractions were reduced in mesenteric arteries but not aortic rings of SW rats. Furthermore, eNOS blockade with L-NAME caused additional contractions in phenylephrine-precontracted mesenteric arteries and the greatest effect was in the SW group, but again there were no differences in aortic rings. Basal NO release was therefore greater in mesenteric arteries but not in aorta from soy-fed rats. The reason for this difference remains unclear, but the lack of effect of soy diet on basal NO in aorta may be reflected in the lack of effect on phenylephrine and K^+PSS - induced contraction amplitude in the same tissue.

We show that in phenylephrine-constricted mesenteric artery, charybdotoxin and apamin further enhanced contraction in the presence of L-NAME, suggesting a small contribution of basal EDHF to tone. The size of this component may have been reduced by the high concentration of phenylephrine used [24], but it was nevertheless similar in both SD and SW, indicating that basal EDHF release was probably unaffected by the soy diet. The agonist-induced EDHF response, however, was enhanced by the soy diet, since we found

that increased sensitivity of phenylephrine-constricted mesenteric arteries from SW rats to ACh persisted in the presence of L-NAME, but was abolished by charybdotoxin and apamin. Differential regulation of basal and agonist-stimulated NO/EDHF release was also observed in an earlier study in rats where L-NAME greatly enhanced both basal resistance and basal mean arterial blood pressure while charybdotoxin and apamin had no effect, whereas both ACh- and bradykinin-mediated increases in mesenteric blood flow were blocked by charybdotoxin/apamin but not by L-NAME or indomethacin [25]. Although charybdotoxin is known to inhibit BK_{Ca} channels (and IK_{Ca} channels), and phenylephrine contractions are influenced by BK_{Ca}, IK_{Ca}, and SK_{Ca} channel activity in the mesenteric circulation [26], BK_{Ca} blockade with iberiotoxin has no effect on ACh-mediated relaxation in third- or fourth-order mesenteric arteries, despite preconstriction with phenylephrine [27]. As a result we suggest that the action of charybdotoxin/apamin on ACh-mediated relaxation is through blockade of IK_{Ca}/SK_{Ca} and therefore of EDHF.

These effects of a soy diet on NO and EDHF responses may be due to the estrogenic actions of the isoflavones genistein and daidzein [8,28], since endogenous estrogens appear to influence NO and EDHF-mediated relaxation [29–31]. Furthermore, ovariectomy has been reported to reduce the EDHF component of endothelium-dependent relaxation and ACh-mediated hyperpolarisation in female rats to levels found in males, an effect reversed by estradiol treatment [30,32]. Moreover, estradiol may alter EDHF activity via changes in expression of connexin-40 and/or 43, components of myoendothelial gap junctions [33,34].

Despite differences in basal NO production, we found that mesenteric contractile responses to phenylephrine differed between SD and SW rats even after blockade of both NO and EDHF, suggesting that nonendothelial factors may also contribute to differences in arterial tone. One possibility is an alteration in agonist receptor expression, since in ovariectomised rats, where contraction to phenylephrine is enhanced, α 1-adrenoceptor expression is reduced by estradiol treatment [35]. However, as K⁺PSS contractions were also larger in SD rats, this suggests that other changes within the smooth muscle may have occurred. These could include enhanced voltage-dependent Ca²⁺ entry [36,37] or vascular remodeling [38].

Effects of SOD and catalase on vascular tone

We found previously that SD rats had reduced expression of antioxidant enzymes Mn-SOD and cytochrome *c* oxidase, increased levels of malondialdehyde, and reduced glutathione levels compared to SW rats [4]. Enhanced superoxide levels in SD rats may inhibit NO-mediated relaxation, since it both scavenges NO and inhibits eNOS [10,39], whereas enhanced SOD expression in SW rats might result in less superoxide and more H₂O₂ (a product of superoxide dismutation). We therefore evaluated the effects of combined SOD/catalase, to destroy both superoxide and H₂O₂, in phenylephrine-contracted aorta and mesenteric artery and PGF_{2 α} -constricted pulmonary artery. In PE-precontracted aortic rings SOD/catalase caused sustained relaxation and this response was diminished in rings from SW rats, suggesting that dietary soy had indeed caused a reduction in oxidative stress. Why this effect was not reflected by a greater effect of L-NAME on phenylephrine contraction in aortae from SW rats is unclear.

In mesenteric arteries SOD/catalase had no effect on the phenylephrine contraction. This was unexpected, since contractile force and basal NO production were both altered by a soy diet. A possible explanation for this finding is that in mesenteric, *but not aortic vessels*, H₂O₂ promotes relaxation. Indeed, exogenous H₂O₂ relaxes phenylephrine-precontracted rat mesenteric arteries independently of nitric oxide synthase and cyclooxygenase [40,41], but is prevented from doing so by depolarisation [40]. H₂O₂ may therefore itself be an EDHF, or it may enhance agonist-mediated EDHF activity [42,43]. If destruction of endogenous H₂O₂ by catalase is indeed modulating EDHF in the mesenteric circulation, then it may oppose the effect of increased basal NO bioavailability due to SOD-mediated destruction of superoxide.

Applying this assumption to agonist-mediated relaxation, the predominantly EDHF-dependent ACh-induced relaxation should be inhibited by SOD/catalase, and indeed we found that it caused a right shift in the ACh concentration response. Furthermore, a similar right shift occurred in the presence of L-NAME, showing that it is indeed the EDHF component of the relaxation that is influenced by SOD/catalase. However, since the combined action of L-NAME and SOD/catalase did not completely abolish the relaxation, H₂O₂ does not account for the EDHF response in its entirety. In any case, we found that SOD/catalase abolished the difference in sensitivity to ACh between SW and SD arteries. Thus the increased expression of Mn-SOD in SW rats [4] may underpin the enhanced ACh-mediated EDHF response in those animals via an increase in endogenous H₂O₂ production [42,43].

We previously reported that switching rats from a soy-deficient diet to a soy rich diet restored ACh-mediated relaxation in rat small pulmonary arteries [4]. EDHF is known to contribute to agonist-induced relaxation in small pulmonary arteries [44], but whether H₂O₂ is an EDHF or modulates EDHF in the pulmonary circulation is unknown. Unlike with mesenteric arteries, H₂O₂ directly constricts pulmonary arteries, but when they are precontracted with agonist, it causes a transient contraction followed by a sustained relaxation [45]. This complex response is the mirror image of our own present observation that SOD/catalase cause a transient relaxation followed by sustained contraction. The greater sustained contraction to SOD/catalase in pulmonary arteries from SW rats may therefore indicate a more effective suppression of contraction by endogenous H₂O₂ in SW arteries.

Hypoxic pulmonary vasoconstriction

HPV is a normal adaptive process whereby pulmonary arteries constrict to hypoxia in order to match regional perfusion with ventilation, but which during global chronic hypoxia may result in pulmonary hypertension and lead to right heart failure. Since sustained HPV is largely endothelium dependent [16] and may be triggered by changes in reactive oxygen species [46], and it and the associated pathologies may be modulated by estrogen [47], we examined the effects of the soy-rich diet on this response. We found a significant decrease in amplitude of the sustained phase of HPV, consistent with a general prorelaxant effect of a soy-rich diet on the pulmonary circulation. However, whether this suppression of contraction is due to changes in ROS production, endothelial function, or to changes in the smooth muscle itself remains to be determined.

Conclusions

Our results suggest that the beneficial effects of dietary soy on cardiovascular function in aging male rats [4] are associated with a generalized reduction in contractile force and improved endothelial function in resistance arteries. The latter involves enhancement of agonist-mediated EDHF activity and increased bioavailability of NO. Our results are also consistent with a prorelaxant role for endogenous superoxide dismutase in mesenteric and pulmonary arteries via both the destruction of superoxide and a concomitant enhancement of endogenous H₂O₂-mediated relaxation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

eNOS	endothelial nitric oxide synthase
EDHF	endothelium-derived hyperpolarising factor
NO	nitric oxide
HPV	hypoxic pulmonary vasoconstriction
SD	soy-deficient diet
SW	soy-rich diet
PSS	physiological salt solution
L-NAME	N ^ω -nitro-L-arginine methyl ester
Ach	acetylcholine
SOD	superoxide dismutase
cat	catalase
eNOS	endothelial nitric oxide synthase
EDHF	endothelium-derived hyperpolarising factor
NO	nitric oxide
HPV	hypoxic pulmonary vasoconstriction
SD	soy-deficient diet

SW	soy-rich diet
PSS	physiological salt solution
L-NAME	N^{ω} -nitro-L-arginine methyl ester
Ach	acetylcholine
SOD	superoxide dismutase
cat	catalase

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Fig. 1. Effect of soy-rich diet on contractile responses in isolated arteries.

(A) Contractions induced by K^+ PSS and $10 \mu M$ phenylephrine (PE) in mesenteric arteries (measured at 7 min). Contraction amplitude was significantly smaller in SW ($n = 10$) compared to SD ($n = 11$) (** $p < 0.01$). (B) Contractions induced by $80 mM$ K^+ PSS and $20 \mu M$ $PGF_{2\alpha}$ in pulmonary arteries, showing a significant reduction in amplitude (* $p < 0.05$) in SW ($n = 14$) compared to SD ($n = 14$). All data expressed as mean $\pm$ SE.

Fig. 2. Effects of soy-rich diet on tonic NO and EDHF activity during phenylephrine-mediated contraction in mesenteric arteries.

(A) Example traces from a mesenteric artery of a SW rat showing the control contraction to 10 μ M phenylephrine (PE, top trace), the effect of L-NAME (applied after 2 min for a further 5 min; 100 μ M; middle trace) and the effect of charybdotoxin (CTX) and apamin (100 nM each, in the continued presence of L-NAME; bottom trace). (B) Contraction amplitude at the end of each control exposure to phenylephrine, or in the additional presence of L-NAME, or of L-NAME plus charybdotoxin and apamin (i.e., after 7 min), in mesenteric arteries from SD rats (solid bars, $n = 11$) or SW rats (open bars, $n = 10$). † $p < 0.01$ vs control within same group (paired t test). * $p < 0.05$, ** $p < 0.01$ vs SD within each treatment. (C) Percentage enhancement of contraction amplitude by L-NAME (left panel) and L-NAME plus CTX/apamin (middle panel), and the charybdotoxin/apamin-specific enhancement (difference between the two treatments, right panel). ** $p < 0.01$, *** $p < 0.001$ vs SD. † $p < 0.05$ vs L-NAME (paired t test). All data expressed as mean $\pm$ SE.

Fig. 3. Acetylcholine (ACh)-mediated relaxation responses in mesenteric circulation.

(A) Relaxation dose responses to ACh (circles), or in the additional presence of 100 μ M L-NAME (squares), and L-NAME in combination with charybdotoxin and apamin (100 nM each, triangles) in mesenteric arteries from SD rats (solid symbols, $n = 11-19$) and SW rats (open symbols, $n = 10-19$). (B) ACh-mediated inhibition of perfusion pressure in phenylephrine-precontracted perfused mesenteric vascular bed from SD (solid circles, $n = 6$) and SW rats (open circles, $n = 5$). All data are expressed as mean $\pm$ SE.

Fig. 4. Effects of acute administration of superoxide dismutase (SOD) and catalase (cat) on phenylephrine (PE)-precontracted rat aortic rings and small mesenteric arteries (MA). (A) Example traces of relaxation response to SOD/cat on phenylephrine-mediated constriction in aortic rings from an SD rat (top trace) and an SW rat (bottom trace). (B) Percentage relaxation induced by SOD/cat in aortic rings and mesenteric arteries from SD rats (solid bars, $n = 12$, $n = 8$ respectively) and SW rats (open bars, $n = 10$, $n = 9$, respectively; * $p < 0.05$ vs SD). (C) Effects of SOD/cat on acetylcholine concentration response (squares) compared to paired controls (circles), in mesenteric arteries from SD rats (solid symbols, $n = 8$) and SW rats (open symbols, $n = 9$). All data expressed as mean $\pm$ SE.

Fig. 5. Acute effects of superoxide dismutase (SOD) and catalase (cat) on PGF_{2α}-precontracted (30 μ M) rat small pulmonary arteries.

(A) Example response in a SW artery showing rapid transient relaxation followed by sustained additional constriction. (B) Average amplitude and time course of these responses in arteries from SD rats (solid circles, $n = 16$) and SW rats (open circles, $n = 14$, * $p < 0.05$ vs SD). (C) Percentage change in amplitude following SOD/catalase application measured at (1) the peak of the relaxation (left panel) and (2) after 10 min of sustained contraction (right panel), in arteries from SD rats (solid bars) and SW rats (open bars, * $p < 0.05$ vs SD). Data expressed as mean $\pm$ SE.

Fig. 6. Effects of soy diet on hypoxic pulmonary vasoconstriction.

(A) Example trace in a pulmonary artery from a SW rat, showing PGF_{2α} preconstriction and superimposed biphasic contractile response to hypoxia (1% O₂). (B) Comparison of responses in arteries from SD rats (solid circles, $n = 11$) and SW rats (open circles, $n = 10$). Data expressed as mean $\pm$ SE.