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Endothelium-dependent vasorelaxation effect of rutin-free tartary buckwheat extract in isolated rat thoracic aorta

Yusuke Ushida[a], Toshiro Matsui[a,*], Mitsuru Tanaka[a], Kiyoshi Matsumoto[a], Hirokazu Hosoyama[b], Atsuhiro Mitomi[b], Yuko Sagesaka[b], Takami Kakuda[b]

[a]*Faculty of Agriculture, Graduate School of Kyushu University, Higashi-ku, Fukuoka 812-8581 Japan*
[b]*ITO EN, LTD., Central Research Institute, Nutrition and Pharmaceutical Laboratory, 21 Mekami, Makinohara, Shizuoka, 421-0516 Japan*

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Abstract

The aim of this study was to point out the potential of tartary buckwheat on vascular functions. A nonabsorbed fraction of hot-water extract of tartary buckwheat on a SP70 column (TBSP-T), which was free from rutin, was used for this aim. In a contractile experiment using Sprague–Dawley rat thoracic aorta rings contracted by 1.0 μM phenylephrine (PE) or 50 mM KCl, TBSP-T evoked a significant vasorelaxation [EC_{50} (mg/ml): PE; 2.2; KCl, 1.9]. By a further fractionation of TBSP-T by liquid–liquid partitioning into basic, neutral and acidic fractions, a marked enhancement of vasorelaxation effect was observed only for acidic fraction (EC_{50}, 0.25 mg/ml). The action of acidic fraction was significantly attenuated in endothelium-denuded aortic rings and in the presence of nitric oxide synthase inhibitor, N^G-monomethyl-L-arginine (100 μM). The fraction also enhanced the cyclic guanosine monophosphate (cGMP) production in aortic rings contracted with PE [cGMP (pmol/mg protein): PE, 7.2±2.3; PE+Acidic fraction, 35±8]. These results indicate that acidic fraction could mediate NO/cGMP pathways, thereby exerting endothelium-dependent vasorelaxation action. In conclusion, tartary buckwheat was proven to regulate vascular tones and have latent acidic candidates except for rutin.

Keyword: Tartary buckwheat; Hypertension; Vasorelaxation; Aorta

1. Introduction

A number of studies have shown that polyphenolic compounds including flavonoids and phenolic acids have diverse physiological functions, such as antioxidant, anti-hyperglycemic and antihypertensive properties [1]. Recent studies have also focused on the prophylaxis effect of cardiovascular disease or atherosclerotic lesion since flavonoid intake was epidemiologically proven to be effective in reducing the risk of chronic disease [2]. This prevalence accelerates the elucidation of their underlying mechanism as well as the survey of phytomedicinal plants that play a physiological role in regulating vascular tone. Duarte et al. [3] demonstrated that a significant blood pressure-lowering effect of quercetin, a flavonoid, in spontaneously hypertensive rats (SHRs) for 5-week treatment was closely related with the enhancement of vasodilator response in isolated aortas and reduced oxidant status (decrease in plasma malondialdehyde levels). Moreover, the fact that in vessels from SHRs, endothelium superoxide anion production was enhanced [4] indicates that some natural compounds having the trapping power of oxygen free radicals from NAD(P)H oxidase action in the endothelium would be a beneficial candidate for preventing the onset of lifestyle-related diseases such as hypertension or atherosclerosis.

Buckwheat, a traditional Asian foodstuff, is said to be one of the candidates responsible for beneficial health effects. A well-recognized effect of buckwheat is a reduction of hypertension risk [5], together with the improvement of diabetes mellitus [6]. An epidemiological study on the genesis of hypertension, in which people in the Mustang District of Nepal who take buckwheat as the main food received lower incidence of hypertension with less than 25% regardless of salt tea (Tibetan tea) drinking habitually [5], provides useful information that

* Corresponding author.
E-mail address: tmatsui@agr.kyushu-u.ac.jp (T. Matsui).

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buckwheat may possess a potential ability to regulate blood pressure. The effect induced by buckwheat has been reported to be due to the improvement of increasing capillary fragility or vascular tone by rutin [7], a characteristic flavonoid in buckwheat. According to the report by Xu et al. [8], rutin possesses a weak relaxation effect on porcine coronary artery among flavonoids. The effect was also defined to be induced by the activation of endothelial nitric oxide (NO) synthetic systems, not by the suppression of Ca^{2+} influx [9].

Owing to the health benefit of rutin, tartary buckwheat (*Fagopyrum tartaricum* Gaertn.) has received so far much attention as a natural physiological functional food because of more rutin than common buckwheat [10]. Contrary to the prevalence of rutin functionality, Yildizoglu-Ari et al. [11] clearly demonstrated that rutin itself had no in vivo antihypertensive effect in intact animals even though it exerted a vasodilating effect in in vitro experiments. The paradoxical result would be also supported by its less intact absorption [12]. Thus, we hypothesized that buckwheat components except for rutin should be present in eliciting vascular relaxation effect. In this study, tartary buckwheat was used for this aim since it contains not only high content of rutin but also different kinds of components including flavonoids [13].

2. Materials and methods

2.1. Materials and reagents

Tartary buckwheat was supplied from ITO EN, LTD. (Shizuoka, Japan). One hundred grams of the roasted ground seeds without bran and shorts was subjected to a 1250-ml hot-water extraction for 10 min (×2). After the centrifugation of the extract at 3500×*g* for 15 min, the supernatant was applied onto an SP70 absorption column chromatography (φ 60×120 mm, Mitsubishi Chemical Co., Tokyo, Japan). By a successive elution with water (1800 ml) and methanol (2000 ml), nonadsorbed fraction (TBSP-T), water-eluted fraction (TBSP-W) and methanol-eluted fraction (TBSP-M) were obtained. Yield of each fraction from 100 g of tartary buckwheat was 6.7, 0.57 and 2.6 g for TBSP-T, TBSP-W and TBSP-M, respectively. As a result of PDA-HPLC (photo-diode array high-performance liquid chromatography) determination, rutin content in each fraction was estimated to be 0, 0.018 and 1.4 g for TBSP-T, TBSP-W and TBSP-M, respectively (see Fig. 1). The PDA-HPLC conditions were as follows: 50 μl of 4.0 mg/ml of each fraction except for rutin (1.0 mg/ml) was applied to the HPLC system (Gulliver Series, JASCO Co. Ltd., Tokyo, Japan) and successive detection from 200 to 600 nm was performed by a PDA detector (photo-diode array, MD-1510, JASCO) on a Cosmosil $5C_{18}$-ARII column (φ 4.6×250 mm, Nacalai Tesque, Kyoto, Japan). The elution was done in a linear gradient mode of MeOH (20–100%, 120 min) at a flow rate of 0.5 ml/min. The monitoring absorbance for rutin was set at 355 nm. For the aim of the present study of elucidating the potential role of buckwheat components except for rutin, we used TBSP-T fraction for further experiments. KCl, phenylephrine (PE), N^G-monomethyl-L-arginine (L-NMMA) and acetylcholine (ACh) were purchased from Wako Pure Chemical Industries (Osaka, Japan). DPPH (1,1-diphenyl-2-picrylhydrazyl) was purchased from Wako Pure Chemical Industries. AAPH (2,2′-azobis(2-methylpropionamidine)dihydrochloride), linoleic acid (LA) and Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylc acid) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical-reagent grade and used without further purification.

2.2. Fractionation of TBSP-T

TBSP-T fraction was separated into basic, neutral and acidic fractions (denoted as TB-B, TB-N and TB-A, respectively) by a liquid–liquid partitioning method with ethyl acetate. Namely, 5 g of TBSP-T dissolved in 25 ml of 50 mM HCl was partitioned with 25 ml of ethyl acetate. The aqueous layer was collected to dryness and used as a TB-B fraction. Fifty milliliters of 5% $NaHCO_3$ solution was then added to the solvent layer. After shaking vigorously, the solvent layer was collected to dryness and used as a TB-N fraction. Finally, the remaining aqueous layer was adjusted pH to 3 with 5 M HCl, followed by the addition of 100 ml of ethyl acetate. The solvent layer was collected to dryness and used as a TB-A fraction. All extracts were lyophilized before using in experiments. Yield of each fraction to TBSP-T was 69%, 5.3% and 1.5% for TB-B, TB-N and TB-A fractions, respectively.

2.3. Preparation of isolated thoracic aorta rings

Preparation of thoracic aorta rings for measurement of isometric vessel tension was performed in our previous study [14]. Namely, male 8- to 9-week-old Sprague–Dawley (SD) rats (SPF/VAF Crj:SD; Charles River Japan, Kanagawa, Japan) weighing 280–300 g were anesthetized with diethyl ether and then exsanguinated. The thoracic aorta was carefully excised and equilibrated for 45 min in physiological salt solution (PSS) buffer (pH 7.4) at 37°C, bubbled with a 95% O_2 and 5% CO_2 gas mixture. The PSS buffer had the following composition (in millimoles): NaCl 145, KCl 5.0, Na_2HPO_4 1.0, $CaCl_2$ 2.5, $MgSO_4$ 0.5, glucose 10 and HEPES 5. After the equilibration, the thoracic aorta was cleaned of adhering fat and connective tissue and was cut into rings 2 to 3 mm in length. In order to examine endothelium-independent vascular response, the endothelium was removed by gently rubbing the luminal surface with a fine needle. The effectiveness of endothelium removal was confirmed by the absence of 100 μM ACh-induced vasorelaxation in PE-contracted aortic rings. All experiments were carried out under the Guidance for Animal Experiments in Faculty of Agriculture and in the Graduate Course of Kyushu University and the Law (No. 105, 1973) and Notification (No. 6, 1980 of the Prime Minister's Office) of the Japanese Government.

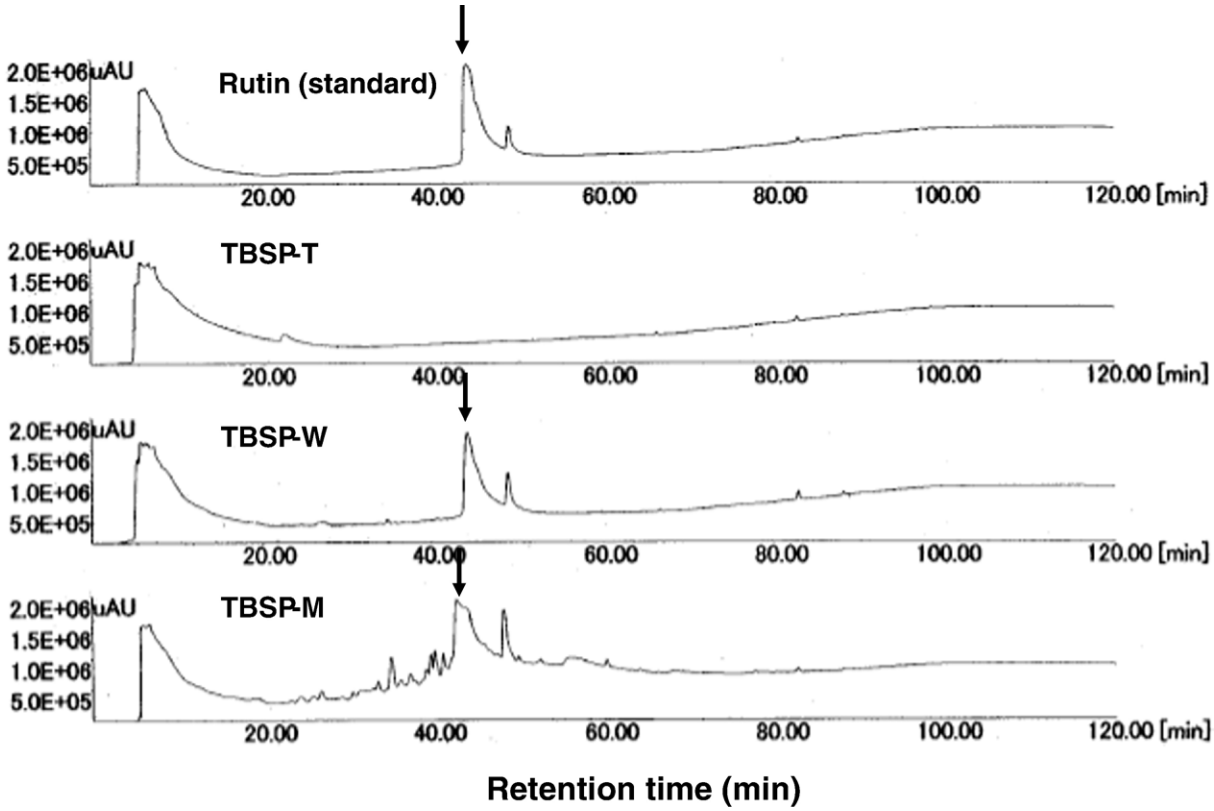

Fig. 1. PDA-HPLC profiles of tartary buckwheat preparations. Hot-water extract of tartary buckwheat was applied onto an SP-70 absorption column chromatography (φ 60×120 mm) to obtain TBSP-T, TBSP-W and TBSP-M. PDA-HPLC conditions were as follows: 50 μl of 4.0 mg/ml of each fraction except for rutin (1.0 mg/ml) was applied to the HPLC system and successive detection from 200 to 600 nm was performed by a PDA detector on a Cosmosil $5C_{18}$-ARII column (φ 4.6×250 mm). The elution was done in a linear gradient mode of MeOH (20-100%, 120 min) at a flow rate of 0.5 ml/min. Arrowed peak shows an elution of rutin monitored at 355 nm.

2.4. Contractive study

Prepared thoracic aorta rings were mounted between two stainless steel hooks in 5-ml organ baths filled with PSS buffer maintained at 37°C. The rings were allowed to equilibrate for 45 min under a resting tension 2 g before experiments were begun. During equilibration period, the PSS buffer adequately bubbled with the gas mixture (95% O_2/5% CO_2) was exchanged every 15 min. Aorta rings were contracted by 1.0 μM PE or 50 mM KCl, and then sample solution was added to the bath in a cumulative manner after maximal contractile tension was achieved. The changes in isometric tension (g) were measured with a force transducer, which was connected via an amplifier (Bridge 8, World Precision Instruments, Berlin, Germany) to a data acquisition system (Biopac System; model MP100, Sta. Barbara, CA, USA). In all experiments for endothelium-dependent vascular responses, special care was taken to avoid damaging the luminal surface of endothelium.

To investigate the role of NO in vascular action of TBSP samples, experiments were performed in the presence of NO synthase (NOS) inhibitor, L-NMMA. Namely, endothelium-intact aorta rings were pretreated with 100 μM L-NMMA for 15 min, followed by the addition of 1.0 μM PE. After achieving a plateau of PE-induced contracted tension, TB-A sample at a dose of 0.34 mg/ml was added and the resulting contracted tension was recorded.

2.5. Measurement of cyclic guanosine monophosphate in aorta rings

Vascular cyclic guanosine monophosphate (cGMP) as a second messenger of NO signaling pathways was evaluated in prepared aorta segments (~20 mg). Aorta rings were incubated in the presence of 1.0 μM PE, 1.0 μM PE+0.34 mg/ml TB-A or 1.0 μM PE+100 μM ACh for 15 min. After incubation, aortic rings were homogenized in PSS containing 5% trichloroacetic acid (TCA). The homogenates were centrifuged for 10 min at 1500×*g*, and the supernatant was used for a cGMP enzyme immunoassay (cGMP EIA, Cayman Chemical Co., Ann Arbor, MI). The pellet was subjected to a protein assay (Bio-Rad Japan, Tokyo, Japan) using bovine serum albumin as standard. The amount of cGMP was expressed as picomole of cGMP/mg protein from aorta ring.

2.6. Radical scavenging assays

Two radical scavenging assays (DPPH and LA oxidation assays) were conducted to evaluate potential ability to trap

radicals by TBSP-T or other fractions. Procedures are as follows. DPPH assay [15]: 200 μl of sample dissolved in ethanol was mixed with 800 μl of 0.1 M Tris–HCl buffer (pH 7.4), followed by the addition of 1 ml of 0.2 mM DPPH solution. After incubation for 30 min, the decrease in absorption at 517 nm was monitored. LA oxidation assay [16]: LA reagent was prepared by adding 11.1 μl of LA to 500 μl ethanol, followed by the addition of 4 ml of sample solution in 0.2 M phosphate buffer (pH 7.4) and the subsequent 150 μl of 1 mM AAPH solution. An aliquot (0.1 ml) of the reagent solution and 30% ammonium thiocyanate was added to 4.7 ml of 75% ethanol. Three minutes after adding 0.1 ml of 20 mM ferrous chloride in 3.5% HCl solution, absorbance at 500 nm was monitored. DPPH radical scavenging activity or LA antioxidative activity was expressed as a Trolox equivalent (TE) by dividing the IC_{50} value of Trolox (7.3±0.2 μg/ml for DPPH assay, 0.0048±0.0008 mg/ml for LA assay) by that of sample.

2.7. Statistics

Results are expressed as mean±S.E. The statistical significance between groups was assessed using a two-way ANOVA followed by Tukey–Kramer's *t* test for post hoc analysis. Statistical differences between two groups were analyzed by unpaired Student's *t* test. $P<05$ was considered to be significant. All analyses were conduced with Stat View J5.0 (SAS Institute, Cary, NC).

3. Results

3.1. Vasorelaxation effect of TBSP-T fraction

In endothelium-intact thoracic aorta rings, TBSP-T fraction containing no rutin demonstrated dose-dependent vasorelaxation effects on both PE- and KCl-contracted aortic rings (Fig. 2A and b). The efficient concentration producing 50% relaxation of maximal contractile response (EC_{50}) by 1.0 μM PE was 2.2 mg/ml, as similar as 1.9 mg/ml for 50 mM KCl contraction. This result provided a first finding that vasorelaxation components except for rutin should be present in tartary buckwheat, playing a potential role in regulating vascular tones.

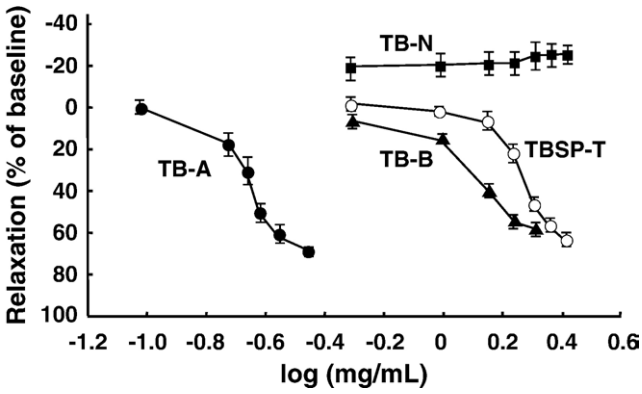

Fig. 3. Relaxation of 1.0 μM PE-contracted thoracic aorta rings by TB-A, TB-N, and TB-B from TBSP-T. Each fraction was added in a cumulative manner to the contracted aortas. Results are expressed as mean±S.E. (n=4–6).

3.2. Vasorelaxation effect of TB-B, TB-N and TB-A fractions

TBSP-T subfractions, TB-B, TB-N and TB-A, were examined for further understanding of vasorelaxation actions induced by TBSP-T fraction. As shown in Fig. 3, only acidic fraction of TBSP showed a significant dose-dependent vasorelaxation effect in endothelium-intact aorta rings contracted with 1.0 μM PE, while less (EC_{50}, 2.0 mg/ml) or no effect was observed for basic (TB-B) and neutral (TB-N) fractions, respectively. A ~9-fold higher improvement of TB-A (EC_{50}, 0.25 mg/ml) in the action compared to TBSP-T was observed, indicating that

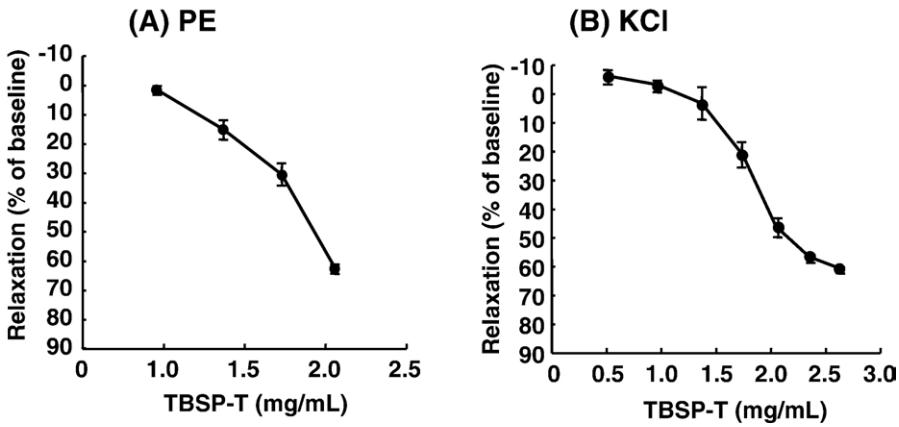

Fig. 2. Relaxation effect of TBSP-T fraction on 1.0 μM PE-contracted (A) and 50 mM KCl-contracted (B) thoracic aorta rings. Resting tension was set at 2 g before stimulation by chemicals. TBSP-T (nonabsorbed fraction of hot-water extract of tartary buckwheat) was added in a cumulative manner (0.5-2.5 mg/ml) to contracted aorta rings. Results are expressed as mean±S.E. (n=4–6). Efficient concentration of TBSP-T fraction producing 50% relaxation of maximal contractile response (EC_{50}) in each contracted aorta ring was estimated from obtained relaxation curve with concentration.

acidic components containing in tartary buckwheat may be responsible for eliciting the vasorelaxation effect.

3.3. Involvement of endothelium in TB-A fraction-induced vasorelaxation action

The vasorelaxation action of TB-A was examined in the endothelium-denuded aorta rings by confirming no vascular response against 100 μM ACh (Fig. 4). As shown in Fig. 4, a complete abolishment of vasorelaxation action of TB-A in endothelium-intact aorta rings was observed by removing endothelium layer from thoracic aorta rings (P=.0123), suggesting that the TB-A fraction may act in an endothelium layer, not in a direct involvement in smooth muscle layer.

3.4. Role of NO/cGMP pathways in TB-A fraction-induced vasorelaxation action

The apparent vasorelaxation effect of TB-A fraction at a concentration of 3.4 mg/ml in endothelium-intact aorta rings significantly failed to evoke in the presence of 100 μM L-NMMA, an eNOS inhibitor (Fig. 5). In 1.0 μM PE-stimulated aorta rings, a basal cGMP level (7.2±2.3 pmol/mg protein) was significantly (P<05) enhanced by the addition of 0.34 mg/ml TB-A fraction (35±8 pmol/mg protein) as well as 100 μM ACh-induced increment as a positive control (Fig. 6). Both results indicate that the vasorelaxation effect induced by TB-A or TBSP-T fraction was closely associated with endothelium-dependent NO/cGMP pathways.

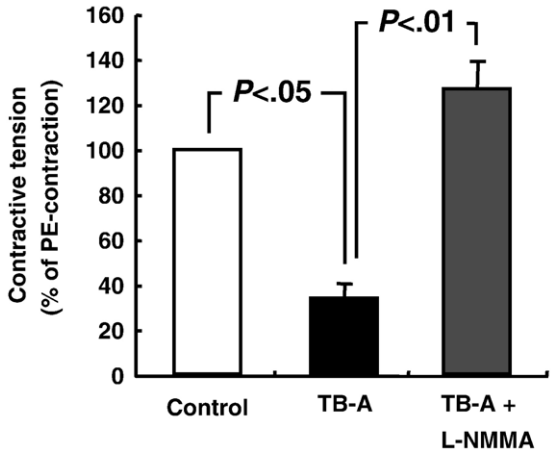

Fig. 5. Relaxation effect of TB-A on 1.0 μM PE-contracted thoracic aorta rings in the presence of 100 μM L-NMMA. L-NMMA was added before PE contraction, and contractive tension was recorded after the addition of 0.34 mg/ml TB-A. Results are expressed as mean±S.E. (n=4–6). Significant difference between each group was evaluated by unpaired Student's t test. P<05 was considered to be significant.

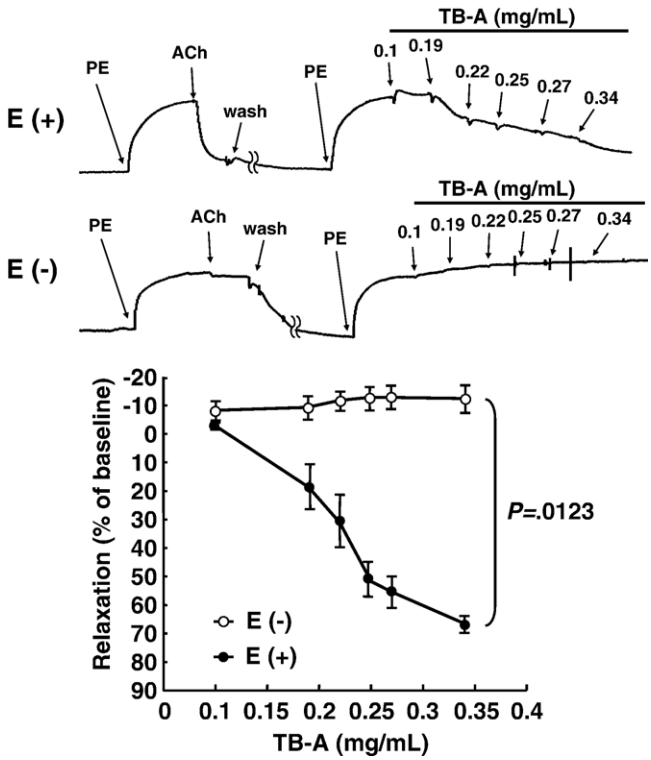

Fig. 4. Relaxation profiles of endothelium-intact (+) or endothelium-denuded (−) aorta rings by acidic fraction (TB-A) and each relaxation behavior. TB-A fraction was added in a cumulative manner (0.1–0.34 mg/ml) to 1.0 μM PE-contracted aortas. Removal of endothelium layer was confirmed by the addition of 100 μM ACh before PE contraction. Results are expressed as mean±S.E. (n=4–6). Significant difference between E (+) and E (−) groups was evaluated by a two-way ANOVA (P=.0123).

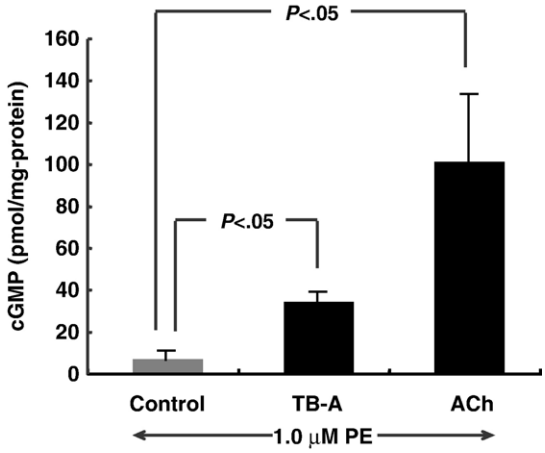

Fig. 6. Effect of TB-A on cyclic GMP level in 1.0 μM PE-stimulated intact thoracic aorta rings. TB-A fraction (0.34 mg/ml) was added to PE-stimulated aortas (~20 mg), and the cGMP level (pmol/mg protein) was determined by a cGMP enzyme immunoassay. ACh (100 μM) was used as a positive control. Results are expressed as mean±S.E. (n=4). Significant difference between each group was evaluated by unpaired Student's t test. P<05 was considered to be significant.

3.5. Radical scavenging activity

DPPH radical scavenging activities of TB-B, TB-N and TB-A fractions were measured as well as LA antioxidant activity. As summarized in Table 1, only acidic fraction of TBSP-T showed an apparent DPPH radical scavenging activity, while no activity was observed for other fractions. However, the DPPH radical scavenging activity of 0.34 TE capacity was still weaker than, for example, green tea extract (2.9 TE capacity) (commercial product from Taiyo Kagaku Co., Mie, Japan) as poor as LA antioxidative activity, indicating that TB-A fraction or overall TBSP-T fraction would possess less antioxidation power. The result also provided useful information that TB-A or tartary buckwheat would not participate in the suppression of NAD(P)H oxidase/superoxide anion radical ($O_2^-\bullet$) production pathways.

4. Discussion

Decreased NO bioavailability as well as endothelium dysfunction is closely associated with the onset of hypertension disease via arterogenic or hypertrophic effect on vascular wall [17]. In this study, we demonstrate the first finding that tartary buckwheat had a potential ability to induce an endothelium-dependent vascular relaxation in rat thoracic aorta rings. The relaxation effect would be involved in the stimulation of NO/cGMP pathways, not in the direct modification of $O_2^-\bullet$ release because of poor DPPH radical scavenging activity of the sample (Table 1). We also found that the candidates responsible for the vasorelaxation effect in aorta rings were acidic compounds from tartary buckwheat. Rutin, a flavonol glycoside compound (quercetin-3β-D-rutinoside), which is abundant in tartary buckwheat, has also been reported to be a candidate for eliciting vascular relaxation [9], but the vasoactive acidic compounds prepared in this study are free from rutin, revealing that other potential vasodilating compounds must be present in the tartary buckwheat. On the contrary, we cannot rule out the possibility that other fractions (TB-B and TB-N) or TBSP extract may be involved in the epidemic antihypertensive effect in humans [5] by, for example, gastrointestinal production of antihypertensive peptides from buckwheat protein [18] since the contribution of TB-A to vasorelaxation effect would be low due to relatively high dose for relaxation (EC_{50}, 0.25 mg/ml, Fig. 3) and low yield of TB-A to TBSP-T extract (1.5%). However, the significant vasorelaxation action of TB-A (acidic and polar compounds present in buckwheat) must be added to latent physiological functions of buckwheat.

So far, it has been reported that some physiologically functional components such as fagopyritols [4], quercitrin or quercetin [9,19,20] were identified as antidiabetic or vasorelaxation components occurring in buckwheat. As similar to our result that buckwheat extract showed a vasorelaxation effect in PE-contracted aorta rings (Fig. 2a), quercetin that is an aglycon of rutin also evoked vascular relaxation in PE-contracted aorta rings via the control of extracellular Ca^{2+} influx [9], not to direct removal of $O_2^-\bullet$ in endothelium layer [20]. Taking into consideration that quercetin is highly hydrophobic, however, the presence of vasoactive quercetin in TBSP-T or the subsequent TB-A fraction should be excluded in this study. Since our prepared TBSP-T was a nonabsorbed fraction of hot-water extract of tartary buckwheat on an SP-70 absorption chromatography, we suggest that new lead components for regulating vascular tones contain in it, like small and polar compounds, apocynin (*o*-methoxy-catechol), which is a naturally occurring NAD(P)H oxidase inhibitor [21]. Bio-guided isolation and identification experiments are now in progress.

PE leads to vascular contraction via the stimulation of α_1-adrenergic receptor. The contraction is achieved by diverse

Table 1
Antioxidant activities of basic, neutral and acidic fractions of tartary buckwheat extracts

Fraction	TE antioxidant capacity [a]	
	DPPH	LA autoxidation
Basic	0.01 (808±12)	NM
Neutral	NI	NM
Acidic	0.34 (21±0.1)	0.0006 (7.7±1.6)
Green tea extract [b]	2.9 (2.5±0.02)	NM
Trolox	(7.3±0.2)	0.0048±0.0008

Numbers in parentheses show the IC_{50} value (mg/ml) in each assay. NM, not measured; NI, not inhibited.

[a] TE is calculated by dividing the IC_{50} value of Trolox by that of fraction.

[b] Commercially available product.

signaling pathways such as suppression of NO production by NAD(P)H oxidase/ERK1/2 cascade or IP_3/PKC cascade, along with Ca^{2+} channel stimulation [22]. Recent study on vascular physiology also pointed out the involvement of Rho/Rho kinase cascade for contraction [23]. In this study, a complete abolishment of relaxation effect of TB-A fraction in the presence of eNOS inhibitor (Fig. 5) as well as the increase in cGMP level (Fig. 6) by TB-A addition to intact aorta rings allowed us to focus on the involvement of TB-A in NO/cGMP pathways. In addition to direct activation of NOS or increase in mRNA NOS expression by TB-A, other possible roles such as inhibitions of NAD(P)H oxidase [21], NO inactivation reaction with $O_2^-\bullet$ (formation of peroxynitrite or others) [24] or tetrahydrobiopterin oxidization [25] may be involved in the enhancement of NO/cGMP pathways by TB-A because of its poor DPPH radical scavenging activity. The result that 50 mM KCl-induced vasocontraction was inhibited by TB-A cumulative additions in rat aorta rings as similar relaxation potency as in PE contraction (Fig. 2) also suggests another possible role of TB-A in the NO production. Namely, high K^+-induced contraction relates to an increase in Ca^{2+} influx through potential-operated Ca^{2+} channels and subsequent feedback inhibition of NO production by PKC activation in endothelium layer [26]. Thus, we could not rule out the role of TB-A in inhibiting IP_3/PKC cascade in the event of endothelium layer. Although inhibition of angiotensin I-converting enzyme (ACE) also promotes NO accumulation [27], poor ACE inhibitory activity of TB-A with IC_{50} of >1 mg/ml (data not shown) would discard the possibility. However, a cumulative effect induced by weak ACE inhibitory action and poor antioxidative activity of TB-A to its relaxation could not be excluded.

Some physiological studies of natural compounds for an alternative medicinal treatment have been conducted to clarify their vasorelaxation action; ginsenosides could improve NO production through regulation of extracellular Ca^{2+} influx into endothelium layer [28]. We also reported a direct inhibition of L-type Ca^{2+} channel by small peptides, which revealed an antiproliferative action of vascular smooth muscle cells [29] as well as vasorelaxation action in KCl-contracted thoracic aorta rings [14]. These reported vasorelaxation mechanisms should be also considered for further study of TB-A-induced vasorelaxation mechanism(s). In addition, animal experiments are now in progress to assess the relevance of the in vitro results to the in vivo situation.

5. Conclusion

The present study demonstrated that tartary buckwheat has a potential ability to regulate vascular tones via the NO/cGMP pathways. The candidates responsible for the effect would be acidic compounds other than the typical buckwheat components, rutin, quercitrin or quercetin.

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