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VASODILATING, SPASMOLYTIC, INOTROPIC AND CHRONOTROPIC ACTIVITIES OF CURCUMINOIDS FROM *CURCUMA LONGA* IN ISOLATED ORGAN PREPARATIONS OF GUINEA PIGS

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Turmeric is a yellowish orange spice, widely used in Asian cuisine and obtained from the rhizome of *Curcuma longa*. It is a mixture of three curcuminoids namely, curcumin, demethoxycurcumin and bisdemethoxycurcumin. Turmeric has been used as a medicinal substance since ancient times for respiratory and gastrointestinal problems. The aim of the present study was to investigate which curcuminoid contributes to the observed pharmacological activities, all three curcuminoids, the major curcumin metabolite tetrahydrocurcumin, and the non-enzymatic curcumin hydrolysis products ferulic acid, feruloyl methane and vanillin were analyzed for spasmolytic, inotropic and chronotropic activity. Furthermore, their uptake in respective tissue samples was also investigated and correlated with activity. Spasmolytic activity was determined in guinea pig ileum, aorta and pulmonary artery. Inotropic and chronotropic activity was determined on guinea pig papillary muscles and right atrium respectively, while tissue uptake was quantified by using high-performance liquid chromatography (HPLC). All the curcuminoids exhibited significant spasmolytic activity with highest EC_{50} values for bisdemethoxycurcumin (5.8 ± 0.6 μM) followed by curcumin (12.9 ± 0.7 μM), demethoxycurcumin (16.8 ± 3 μM) and tetrahydrocurcumin (22.9 ± 1.5 μM). While only demethoxycurcumin was able to significantly relax the pulmonary artery with EC_{50} value of 15.78 ± 0.85 μM. All three curcuminoids showed mild negative chronotropic effects in the isolated right atrium; tetrahydrocurcumin demonstrated no activity. Curcumin and bisdemethoxycurcumin also showed mild positive inotropic effect whereas demethoxycurcumin and tetrahydrocurcumin exhibited weak negative inotropic one. Interestingly, ferulic acid, feruloyl methane and vanillin demonstrated no pharmacologicical activity at all in the various isolated organs. All three curcuminoids and tetrahydrocurcumin showed high uptake into the various tissues where concentrations correlated with pharmacological activity. The results indicate pronounced differences in the *in vitro* pharmacological activities of curcumin, demethoxycurcumin, bisdemethoxycurcumin and tetrahydrocurcumin which have to be considered in humans after per-oral intake of turmeric powder.

Key words: *curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin, spasmolytic activity, guinea pig*

INTRODUCTION

Turmeric is a yellowish-orange spice obtained from the rhizome of *Curcuma longa* (*C. longa*). It is a mixture of three phenolic compounds collectively called curcuminoids, which consist of mainly curcumin and smaller amounts of demethoxycurcumin and bisdemethoxycurcumin (1). Around the world turmeric is used as a spice, colorant, flavorant and condiment. Beside its culinary value it is also widely used by traditional healers since the ancient times for anemia, cough, fever, pain, jaundice, wound healing, insect bite, itching, eczema, liver disorders, urinary diseases and joint problems (2, 3). In Chinese herbal medicine curcuma is traditionally used against various syndromes caused by the obstruction of blood circulation like psychataxia and arthralgia (4). It is also used against diarrhea, flatulence, gastritis, gastroesophageal reflux disease, asthma, cough and cold (2, 5-7). These traditional uses indicate antispasmodic and/or smooth muscle relaxant effects of curcuma drugs which are well corroborated by pharmacological studies. Curcuma extracts from different species relaxed pre-contracted aorta independent of NO synthesis (4), while the extract prepared from the rhizomes of *C. longa* exhibited spasmolytic effect on rabbit intestinal preparation with the indications for calcium channel blocking activity (2). Sodium salt of curcumin reduced blood pressure and heart rate in anesthetized dogs and cats when administered intravenously. This hypotensive and bradycardic effect was not antagonized by pretreatment with propranolol, mepyramine, atropine or bilateral vagotomy thus excluding β-adrenergic, histaminergic, muscarinic or vagal nerve involvement (8). The blood pressure lowering effect and bradycardia was also reported in conscious rats by methanolic extract of *C. longa* which was attributed to calcium channel blockade (9). It also exerted spasmolytic effect on smooth muscles of guinea pig vas deferens and intestine of dogs (8).

Since, crude curcumin is a mixture of three curcuminoids and it is still unknown which specific compound contribute to the spasmolytic activity. Curcumin itself is sensitive to oxygen, UV and visible light and quite unstable at physiological pH (10). When curcumin was added to 0.1 M phosphate buffer, pH 7.4, curcumin was stable after 1 hour and then started degrading gradually. Almost 50% and 90% of curcumin is degraded after 3 and 8 hours of incubation, mainly forming ferulic acid, feruloyl methane and vanillin due to non-enzymatic hydrolysis (*Fig. 1*) (11). In comparison to curcumin, demethoxycurcumin and bisdemethoxycurcumin are much more stable (1). Interestingly, the instability was dependent on the concentration of the curcuminoids and was most pronounced at low concentrations (11). Moreover, curcumin have extensive intestinal metabolism (12) and in body it is rapidly metabolized by alcohol dehydrogenase into dihydrocurcumin, octahydrocurcumin (minor metabolites) and tetrahydrocurcumin, (major metabolite). These reductive metabolites are extensively conjugated with glucuronic acid and sulfuric acid and rapidly excreted into feces (1). Up to now, only few studies have reported about the spasmolytic activity of a turmeric extract containing curcumin, demethoxycurcumin and bisdemethoxycurcumin (13). Whether curcumin is the only active compound or demothoxycurcumin and bisdemethoxycurcumin, the main curcumin metabolite tetrahydrocurcumin as well as the curcumin degradation products ferulic acid, feruloyl methane and vanillin may also contribute to spasmolytic activity is not known yet. Thus, in the present study we investigated for the first time the spasmolytic activity of curcumin, desmethoxycurcuminoids, tetrahydrocurcumin and the three curcumin degradation products on guinea pig aorta, ileum, papillary muscle, pulmonary artery and right atria. As pharmacological activity is strongly dependent on tissue concentration, we also determined for the first time the tissue uptake of these compounds into different tissue preparations by a sensitive high-performance liquid chromatography (HPLC) assay and correlated uptake with observed pharmacological effects.

MATERIALS AND METHODS

Chemicals

Curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin, ferulic acid, vanillin and feruloyl methane were purchased from Sigma-Aldrich (Munich, Germany (purity: ≥ 98.0%). Methanol and water were of HPLC grade and obtained by Merck, Darmstadt, Germany. All other chemicals and solvent were of analytical grade, commercially available, and used without further purification.

Experiments on isolated tissue preparations

Guinea pigs of either sex weighing 340 – 480 g were obtained from the Department of Laboratory Zoology and

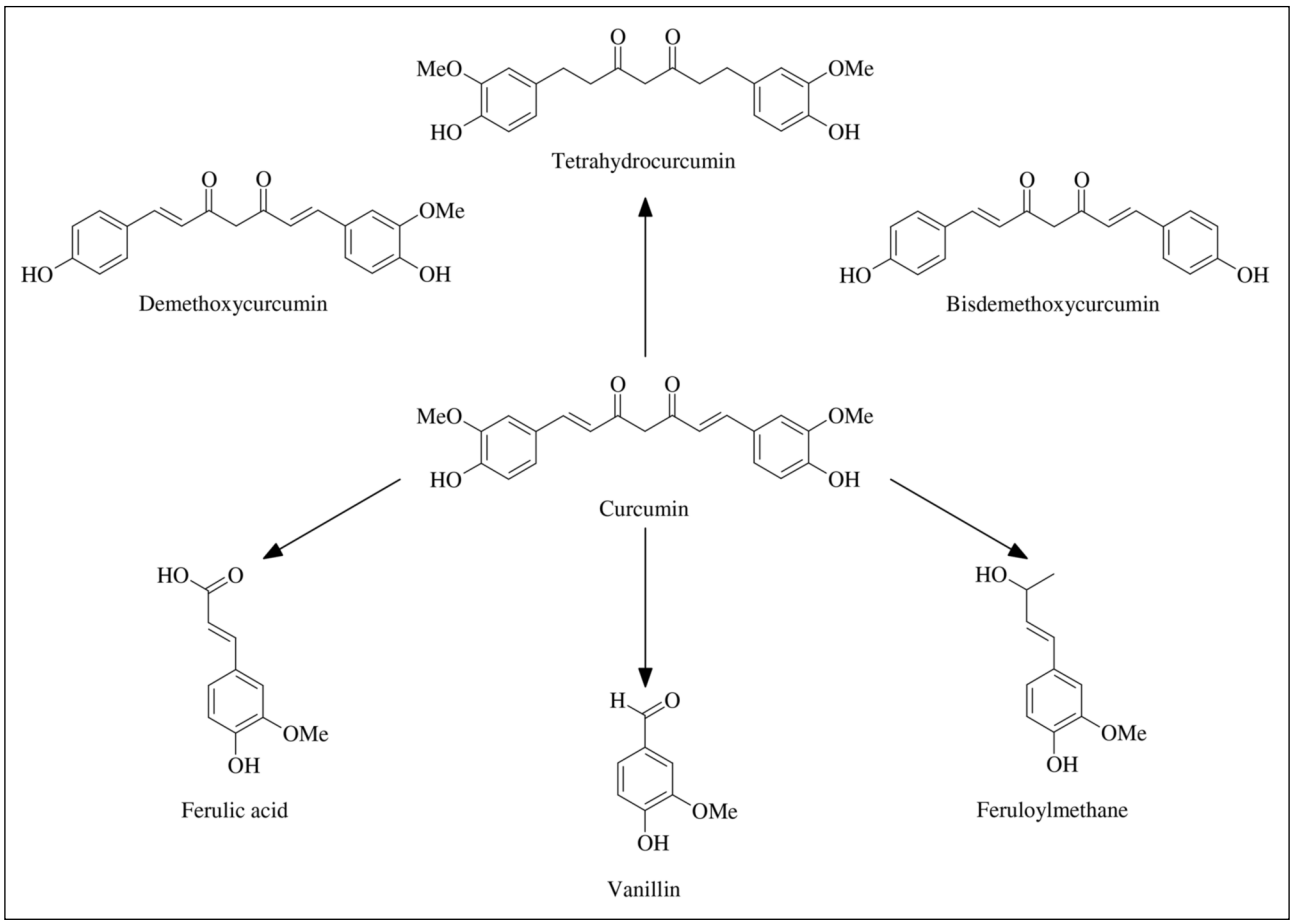

Fig. 1. Chemical structures of the three curcuminoids curcumin, demethoxycurcumin, bisdesmethoxycurcumin, the curcumin metabolite tetrahydrocurcumin and the curcumin degradation products ferulic acid, feruloyl methane and vanillin.

Genetics, Medical University, Himberg, Austria. Animals were kept in air-conditioned room at a temperature of 22 – 24°C and relative humidity 50 – 60% with 12 hour photo period. On the day of experiments animal was sacrificed by a blow on the neck followed by, animal heart, aorta, pulmonary artery and ileum were surgically excised and kept in Krebs-Henseleit solution (NaCl 144.9 mM, KCl 4.73 mM, $CaCl_2$ 3.2 mM, $MgSO_4$ 1.18 mM, $NaHCO_3$ 24.9 mM, KH_2PO_4 1.18 mM and glucose 10 mM; pH 7.2 – 7.4), continually aerated with 95% O_2 and 5% CO_2. Papillary muscles were dissected from the right ventricle of heart and cleared from Purkinje fibers to avoid spontaneous activity. We used muscles having diameter less than 0.87 mm to ensure proper oxygen supply. The right atrium was also dissected to check the chronotropic activity. Both aorta and pulmonary artery were cleaned and rings of 5 mm were cut while ileum was cut from the terminal portion into pieces of 1 – 2 cm. One end of the dissected tissues was tied with silver wire for attachment with tissue holder while the other end was connected with force transducer (Transbridge™, 4-Channel Transducer Amplifier, World Precision Instruments, Sarasota, FL, USA). Terminal ileum was contracted by 60 mM KCl while pulmonary artery and aorta rings with 90 mM KCl solution which produce sustained contractions in respective tissues. The test conditions might be therefore stated as 'vasodilatory effect during high K-pre-constricted state'. Papillary muscles were electrically stimulated by an Anapulse Stimulator model 301-T and an Isolation UnitModel 305-1 (WPI, Hamden, CT, USA) with rectangular pulses of 3 ms at a frequency of 1 Hz. The amplitude of stimulation pulse was kept 10% above the threshold level. To obtain maximum contractility from the respective tissues, a constant resting tension of 3.9 mN for papillary muscle, 4.9 mN for terminal ileum, 10.4 mN for right atrium and 19.6 mN for aorta and pulmonary artery rings was applied throughout the experiment. After a control period of 15 min, different concentrations of test compounds were applied cumulatively in a bath solution every 30 min until steady effect was obtained. The responses were recorded by a chart recorder (BD 112 Dual Channel, Kipp & Zonen) and evaluated later. Stock concentrations for test compounds were made with distilled water and where required with DMSO. To exclude the effect of DMSO, experiments were performed with solvent only and observed effect was subtracted from the response of the test compounds.

Tissue uptake and high-performance liquid chromatography (HPLC) analysis

For uptake experiments tissue samples were incubated in Krebs-Henseleit at 37°C containing 100 µM curcumin, desmethoxycurcumin and bisdesmethoxycurcumin and tetrahydrocurcumin, respectivity. After 30 min, tissue samples were washed 5 times with ice cold Phosphate buffer saline (PBS) and subsequently homogenized by ULTRA TURAX® homogenizer and diluted three times with PBS followed by centrifugation at 13,500 g for 10 min (4°C). Supernatant was carefully collected for detection and quantification of compounds on HPLC as described previously with some minor modifications (14). For HPLC analysis a Dionex UltiMate 3000 system equipped with an L-7250 injector, an L-7100 pump, an L-7300 column oven (set at 35°C), a D-7000 interface, and an L-7400 UV detector (Thermo Fischer Scientific) set at the wavelength of 420 nm for curcumin, demethoxycurcumin, bisdemethoxycurcumin and 280 nm for tetrahydrocurcumin was used. Separation of curcuminoids and their metabolite tetrahydrocurcumin was carried out at 35°C with the help of Hypersil BDS-C18 column (5 µm, 250 × 4.6 mm I.D., Thermo Fischer Scientific), followed by Hypersil BDS Precolumn (5 µm, 10 × 4.6 mm I.D.). The mobile phase consisted of a continuous linear gradient, mixed from 10 mM ammonium acetate/acetic acid buffer, pH 5.0 (mobile phase A) and methanol (mobile phase B), having a flow rate of 1 mL/min. A filter (0.45 µM, HVLP04700; Millipore) was used for mobile phase filtration. The gradient was ranged from 10 – 90% methanol from 0 – 17 min followed by another increase at 18 min, and then it remained constant till 25 min. Subsequently, the percentage of methanol was decreased within 2 min to 10% for equilibrating the column for 8 min before administration of the next sample. External standard method was used for calibration of chromatogram. Linear calibration curves were performed with standard solution of the compounds, with a concentration range of 0.01 – 10 µg/ml (average correlation coefficients > 0.999). Coefficients of accuracy and precision of these compounds were < 11%.

Statistical analysis

For statistical analysis mean and standard error of mean (SEM) was calculated for 'n' experiments and significance was determined by applying student's t-test for paired values (Sigma Plot version 9.0). Two-way ANOVA was used in order to evaluate statistical significance in a group and between groups of tissue samples from different organs for uptake studies by using GraphPad Prism 7.

RESULTS

Spasmolytic activity on guinea pig ileum

In order to identify and compare the spasmolytic activity of curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin, ferulic acid, feruloyl methane and vanillin we used guinea pig ileum, pre-contracted by 60 mM KCl solution. All curcuminoids but not the degradation products ferulic acid, feruloyl methane and vanillin exhibited significant spasmolytic activity in a concentration dependent manner with EC_{50} values of 12.9 ± 0.7 µM for curcumin, 16.8 ± 3 µM for demethoxycurcumin, 5.8 ± 0.6 µM for bisdemethoxycurcumin and 22.9 ± 1.5 µM for tetrahydrocurcumin (*Fig. 2A*). To rule out the involvement of NO in curcuminoid mediated spasmolytic activity (15), we inhibited endothelial nitric oxide synthase (eNOS) in pre-contracted terminal ileum by incubating with 100 µM nitro-L-arginine (L-NNA) for 45 min. After this incubation period curcuminoids and their metabolite tetrahydrocurcumin was administered in a bolus dose of 20 µM to investigate any effect on spasmolytic activity. The inhibition of eNOS does not exhibit any significant effect on spasmolytic activity (*Fig. 2B*).

Vasodilating activity on pulmonary artery and aorta

The vasodilating potency of curcuminoids and their metabolite was assessed on pulmonary artery and aorta pre-contracted by 90 mM KCl solution. Demethoxycurcumin significantly alleviated the spasm of pre-contracted pulmonary artery with EC_{50} value of 15.78 ± 0.85 µM. Contrary to demethoxycurcumin, curcumin, bisdemethoxycurcumin and tetrahydrocurcumin only modestly dilated the pulmonary artery with a relative vasodilating potency of 38.5 ± 2.8%, 24.3 ± 6.8% and 37.6 ± 8.9%, respectively, indicating that demethoxycurcumin is primarily responsible for relaxation of pulmonary artery (*Fig. 3A*). In pre-contracted aorta tissue rings, demethoxycurcumin, bisdemethoxycurcumin and tetrahydrocurcumin declined the spasm by 35.1 ± 9.6%, 18.6 ± 5.6% and 27.9 ± 7.2% at 100 µM concentration, while no effect

was observed for curcumin (*Fig. 3B*), ferulic acid, feruloyl methane and vanillin.

Inotropic activity

Inotropic activity was performed on papillary muscles. Demethoxycurcumin and tetrahydrocurcumin showed negative ionotropic activity of 23.5 ± 8.9% and 29.2 ± 2.6% respectively, curcumin demonstrated a positive ionotropic effect in papillary muscles of 28.9 ± 8.9%. Interestingly, bisdemethoxycurcumin initially showed ionotropic activity of about 20% at 30 µM which was reduced to 15.2 ± 5.6% at 100 µM (*Fig. 4*). The degradative products ferulic acid, feruloyl methane and vanillin did not exhibit any activity.

Chronotropic activity

Chronotropic activity was determined on isolated guinea pig right atrium. Curcumin, demethoxycurcumin and bisdemethoxycurcumin exhibited a negative chronotropic activity with a reduction of 27.6 ± 4.5%, 35.5 ± 5.2% and 13.4 ± 5.6% in beating frequency at 100 µM (*Fig. 5*). In contrast, tetrahydrocurcumin, ferulic acid, feruloyl methane and vanillin did not exhibit any chronotropic activity.

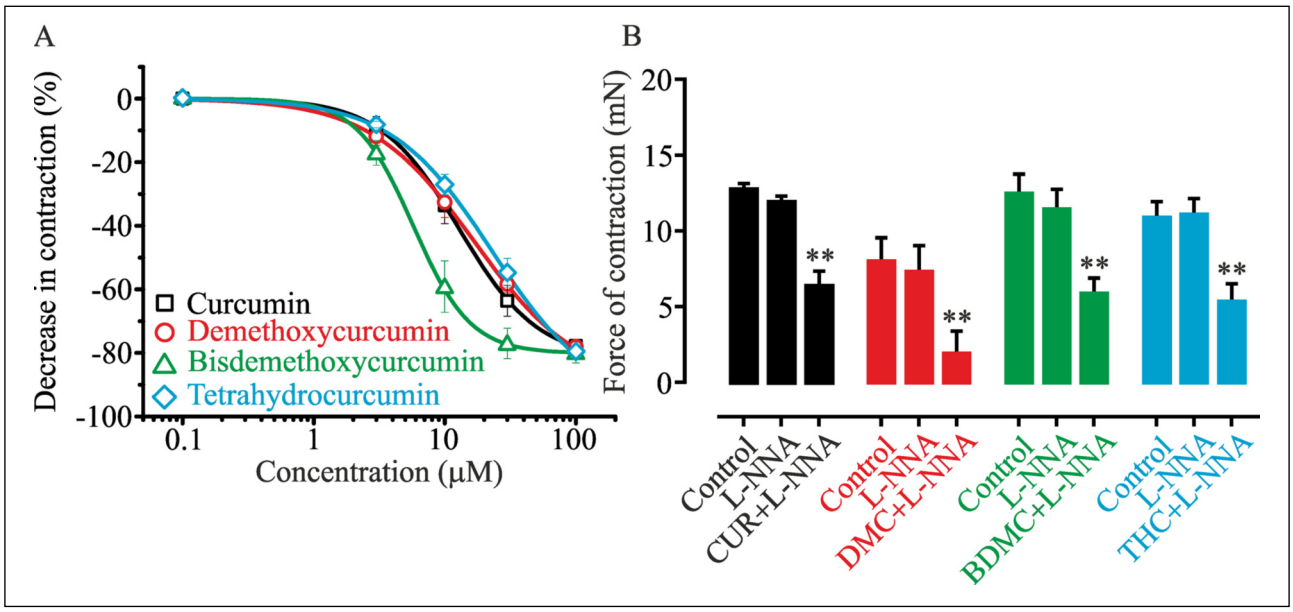

Fig. 2. Spasmolytic activity of curcuminoids and their metabolite. (*A*), concentration response curves for curcumin, demethoxycurcumin, bisdemethoxycurcumin and tetrahydrocurcumin determined on isolated guinea pig terminal ileum. Data is presented as Mean ± SEM (n = 4 – 5). (*B*), bar graph representing the spasmolytic activity of curcumin (CUR), demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC) and tetrahydrocurcumin (THC), after 45 min incubation with L-NNA. Data is presented as Mean ± SEM (n = 4) and significance was determined by applying 2-way ANOVA followed by Tukey´s post-test.

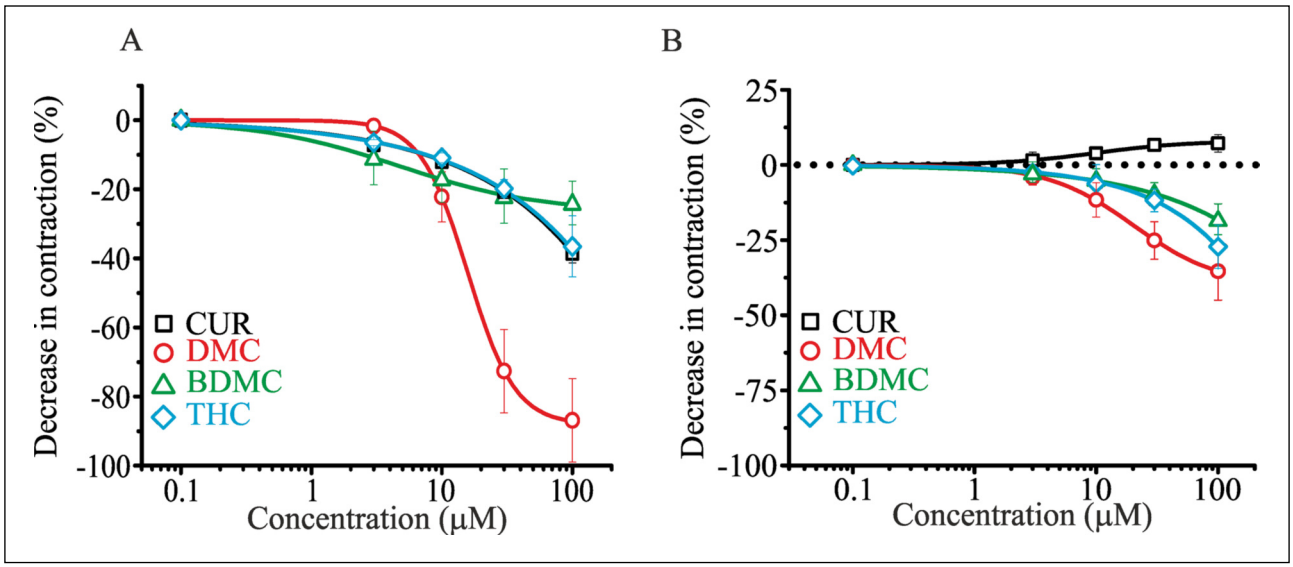

Fig. 3. Vasodilatory activity of curcuminoids and their metabolite. Concentration response curve for curcumin (CUR), demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC) and tetrahydrocurcumin (THC), determined on guinea pig (A) pulmonary artery (n = 5), and (B) aorta (n = 5 – 6). Data is represented as Mean ± SEM.

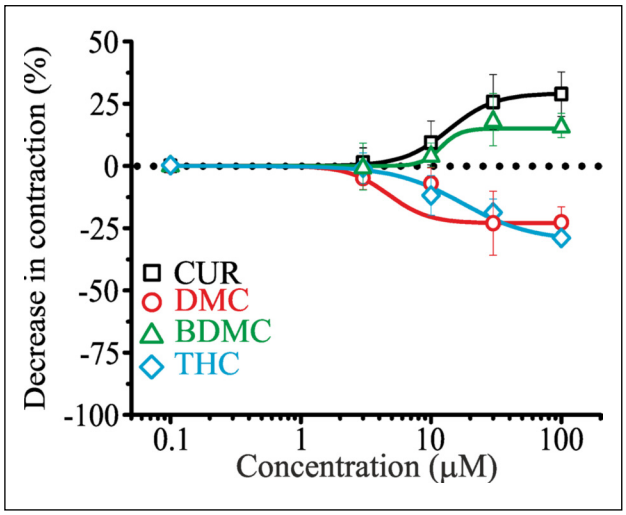

Fig. 4. Inotropic activity of curcuminoids and their metabolite. Concentration response curve for curcumin (CUR), demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC) and tetrahydrocurcumin (THC), determined on papillary muscle from guinea pig right ventricle. Data is represented as Mean ± SEM (n = 4).

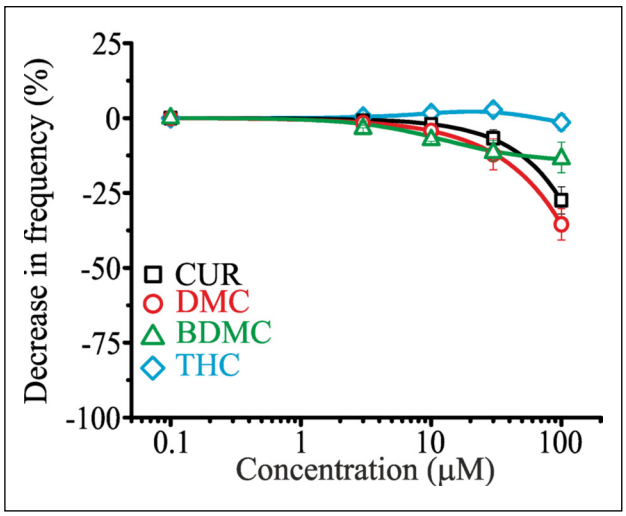

Fig. 5. Chronotropic activity of curcuminoids and their metabolite. Concentration response curves for curcumin (CUR), demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC) and tetrahydrocurcumin (THC), determined on guinea pig right atria. Data is represented as Mean ± SEM (n = 4).

Tissue uptake

For uptake experiments tissue samples of the terminal ileum, aorta, pulmonary artery, right atria and papillary muscles were incubated at 37°C with 100 μM curcumin, demethoxycurcumin and bisdemethoxycurcumin tetrahydrocurcumin, respectively. After 30 min tissue samples were washed 5 times with PBS and immediately homogenized and analyzed by HPLC. As shown in *Fig. 6*, our HPLC assay allowed the clear separation of curcumin (tr = 20.63 min), desmethoxycurcumin (tr = 19.60 min), bisdesmethoxycurcumin (tr = 18.39 min) and tetrahydrocurcumin (tr = 17.74 min). Furthermore, all three curcuminoids and tetrahydrocucumin demonstrated sufficient stability at physiological pH at least for 30 min as no further peaks from metabolites or degradation products showed up in the chromatograms (*Fig. 6*). The two peaks at 3.2 and 3.8 min in the chromatogram of tetrahydrocurcumin are impurities from the incubation medium and only seen at 280 nm and not at 420 nm, which was used for the detection of the other three curcuminoids.

Uptake of curcuminoids and tetrahydrocurcumin strongly differs in the various tissue samples (*Fig. 7*). While bisdemethoxycurumin concentration was highest in the terminal ileum, desmethoxycurcumin showed the highest uptake by the pulmonary artery. In the aorta, demethoxycurcumin and bisdemethoxycurcumin levels were high and at about in the same amount. This was also true for the papillary muscle and right atrium which also showed the highest uptake for demethoxycurcumin (*Fig. 7* and *Table 1*). As ferulic acid, feruloyl methane and vanillin did not show any activity in the isolated organ model no uptake experiments were carried out.

DISCUSSION

Turmeric powder is a commonly used spice in Asian cuisine and obtained from *C. longa*. It contains 40.36 mg/g of curcuminoids with the relative proportion of each compound is approximated as 4.18 – 22.8 mg/g of curcumin, 1.08 – 9.26 mg/g of demethoxycurcumin and 0.40 – 9.50 mg/g of bisdemethoxycurcumin (16). In Southeast Asia daily dietary consumption of turmeric powder is approximated as 1.5 g/day. Turmeric powder is also available as a popular over-the-counter food supplement with the high doses up to 10 g/day. Many of these supplements use piperine in combination with turmeric powder which can increase bioavailability of curcumin in humans up to 2000% (17). Curcuminoids are relatively safe compounds as observed in dose escalation studies, where a consumption of single dose 12 g curcumin by healthy human volunteers did not exhibit any serious side effects (18). Up to now, there are only few data in the literature about the pharmacological activities of the three curcuminoids. Recent *in vitro* studies showed that the potency of demethoxycurcumin and bisdemethoxycurcumin to modulate inflammatory- and cell-proliferating signaling *via* suppression of tumor necrosis factor (TNF)-induced nuclear factor-kappaB (NF-kappaB) activation was only slightly lower than curcumin in various human cancer cell lines (19). Tetrahydrocurcumin, a major metabolite of curcumin, was also shown to possess various biological activities. For example, tertrahydrocurcumin inhibited lipoxygenase to the same extent as curcumin (IC_{50}: 1 μM), (20) and was more than 3-fold more active than curcumin in relieving the sciatic nerve injury of rats (21).

The three curcumin degradation products ferulic acid, feruloyl methane and vanillin were also shown to demonstrate pharmacological activity. So was the free radical scavenging properties of ferulic acid, which was about 7-fold higher compared to curcumin (22). Vanillin is capable of attenuating cancer metastasis by modulating angiogenesis in A549 lung cancer cells (23) and has been shown to have minor anti-inflammatory effects *via* inhibition of cyclooxygenase 2 (COX-2) (24).

Therefore, in the present work, we investigated the spasmolytic activity of three curcuminoids and their major metabolite tetrahydrocurcumin along with degradative products ferulic acid, feruloyl methane and vanillin on guinea pig ileum, aorta and pulmonary artery. We also evaluated the chronotropic and inotropic activities of these compounds on isolated right atrium and papillary muscles respectively. Beside these biological activities we quantified the uptake of curcuminoids and tetrahydrocurcumin in respective tissue samples and compared it with biological activities.

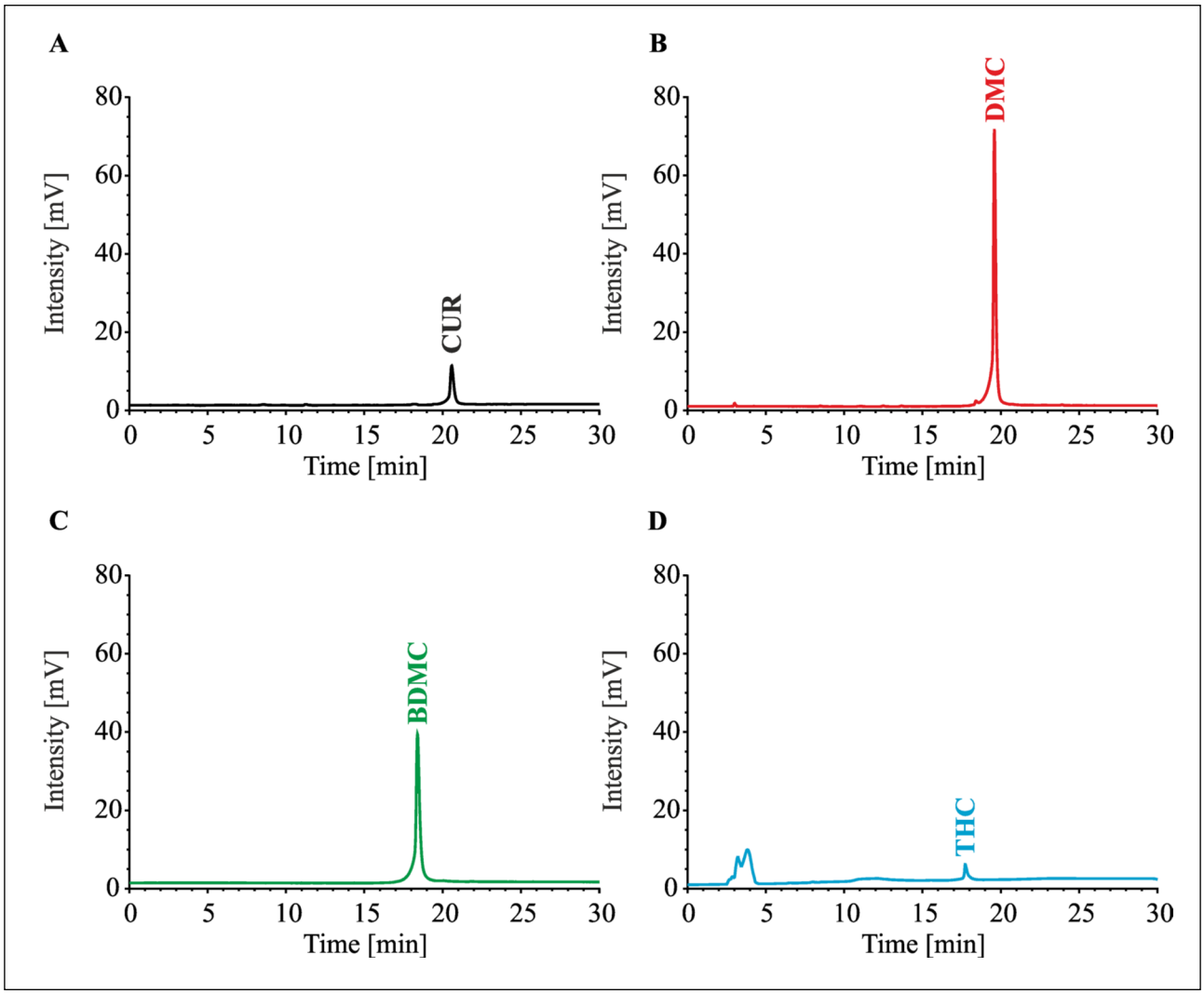

Fig. 6. Representative HPLC chromatograms of curcumin (CUR) (A), demethoxycurcumin (DMC) (B), bisdemethoxycurcumin (BDMC) (C) and terahydrocurcumin (THC) (D) in the pulmonary artery after 30 min at 37°C with 100 µM of the pure compounds.

In isolated organ preparations of ileum all compounds exhibited significant spasmolytic activity (*Fig. 2A*), thus confirming the beneficial effects of turmeric powder against gastrointestinal spasm like irritable bowel syndrome (25, 26). In a pilot study of 105 irritable bowel syndrome patients consuming 144 mg of turmeric extract for 8 weeks, abdominal pain and discomfort was reduced in 25% of the patients (27). As nitric oxide has been described to modulate gastrointestinal movements (28, 29), we assessed the effect of eNOS blockade by L-NNA on curcuminoid mediated spasmolytic activity. However, our experiments demonstrated that blockade of eNOS does not affect the spasmolytic activity of curcuminoids and their metabolite (*Fig. 2B*). The observed spasmolytic effect of curcuminoids may be therefore attributed to a blockade of calcium influx from voltage-gated calcium channels (9, 30, 31), since plant materials relaxing high potassium induced contractions possess calcium channel blocking activity (32-35). In a further experimental setting, curcumin, bisdemethoxycurcumin and tertrahydrocurcumin only modestly whereas demethoxycurcumin significantly relaxed KCl-induced contractions in pulmonary artery with EC_{50} value of 15.78 ± 0.85 µM (*Fig. 3A*). These finding indicates that demethoxycurcumin and not the other curcuminoids are mainly responsible for the vasorelaxation of *C. longa* extracts (9). This effect of demethoxycurcumin on pulmonary artery can be additionally attributed to the inhibition of phosphodiesterase-5 as observed in rat pulmonary artery where only demethoxycurcumin inhibited phosphodiesterase-5 and produced strong vasorelaxation while curcumin and bisdemethoxycurcumin produced mild vasorelaxation by interfering with calcium ion movement (36). In aorta tissue preparation, curcumin showed mild vasoconstriction whereas demethoxycurcumin, bisdemethoxycurcumin and tetrahydrocurcumin exhibited vasorelaxant effect (*Fig. 3B*). This explains the previously reported dual effect of *C. longa* crude extract on aorta rings, where it showed weak vasoconstriction in the absence of any agonists and vasorelaxation in agonist- induced contractions (2, 31). Recent data demonstrated that low concentrations of curcumin (< 5 µM) stimulates the expression of COX-2 mRNA and protein in human coronary artery endothelial cells (37). Furthermore, it also increased the expression of prostaglandin I_2 (PGI_2) and prostaglandin E_2 (PGE_2) synthase mRNA with resultant enhancement of the production of PGE_2 and PGI_2 when adequate amounts of arachidonic acid were present (37). As PGI_2 is a potent vasodilator (38), PGI_2 might contribute, at least

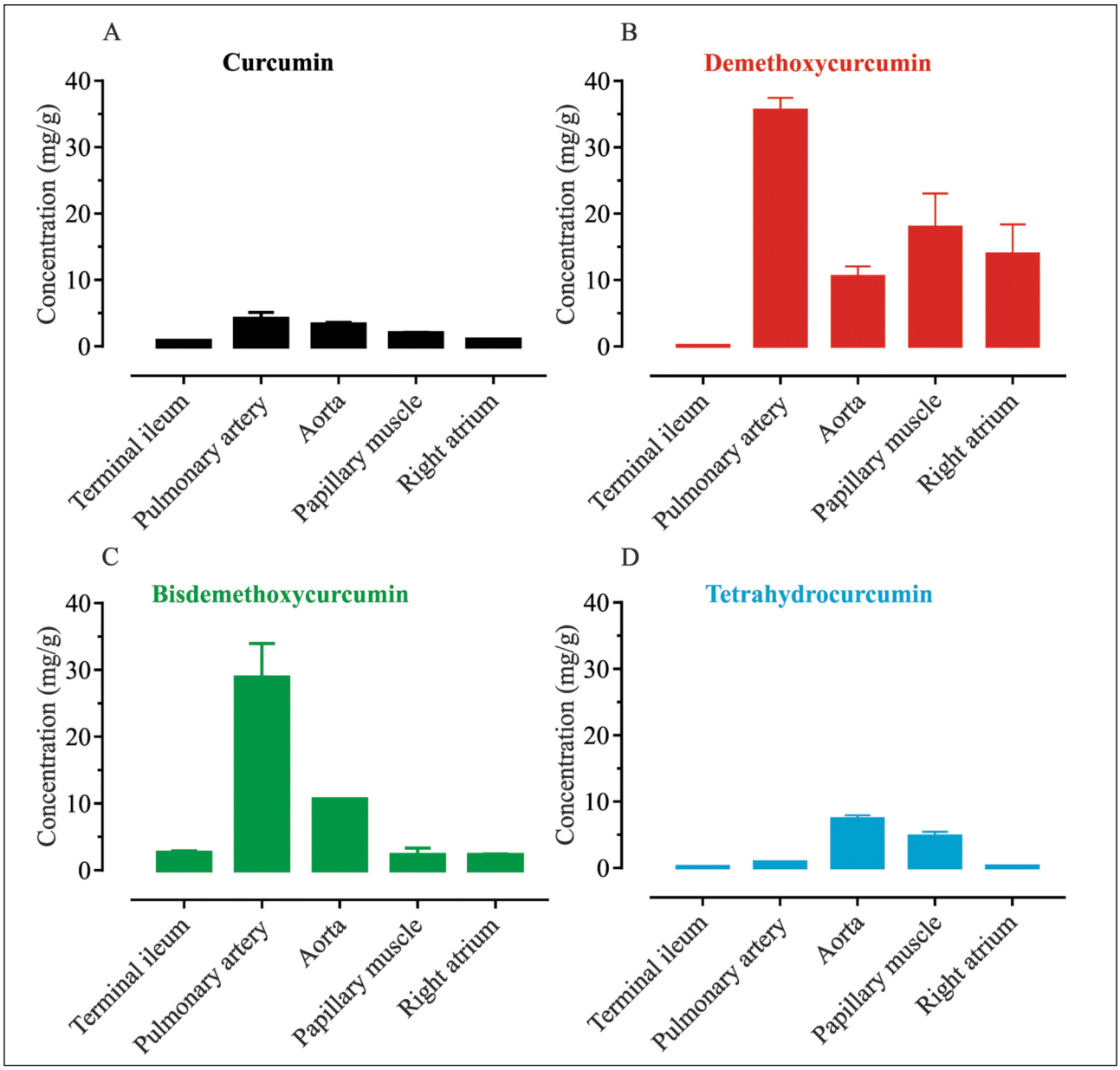

Fig. 7. Tissue uptake experiments for curcuminoids and their metabolite. Bar graphs representing uptake of (A), curcumin (B), demethoxycurcumin (C), bisdemethoxycurcumin (D), tetrahydrocurcumin in respective tissue samples. Data is presented as Mean ± SEM (n = 3).

partly, to the observed vasorelaxant property of demethoxycurcumin and bisdemethoxycurcumin in the isolated aorta rings of guinea pigs. Crude extract of *C. longa* has been reported to exhibit a variable response including both a hypotension and hypertension on arterial blood pressure in anesthetized rats (2). So, we also screened for both inotropic and chronotropic effect of individual curcuminoids and their metabolite tetrahydrocurcumin in right atrium and papillary muscles. All the compounds mildly suppressed rate of atrial contractions thus exhibiting a bradycardic effect (*Fig. 5*). However, in papillary muscle preparation both curcumin and bisdemethoxycurcumin exhibited mild positive inotropic effect whereas demethoxycurcumin and tetrahydrocurcumin showed mild negative inotropic effect (*Fig. 4*). This explains why previously a variable response both (hypotensive and hypertensive) was observed by crude extract of *C. longa* (2). Our results are also in line with a more recent study which observed that curcumin lead to a short time (3 ± 1 min) hypotensive response in non-anesthetized rats and a more prolonged (15 ± 1 min) bradycardic effect (9). This indicates the antagonizing effect of curcuminoids may be beneficial in normalizing the blood pressure. However, pharmacokinetic interactions have been reported after concomitant use of curcuminoids with cardiovascular drugs. Cautions should be therefore taken especially after a high dose of curcuminoids (39, 40).

Uptake of curcuminoids and of tetrahydrocurcumin into various tissue samples correlated with pharmacological activities. Bisdemethoxycurcumin not only demonstrated the most potent spasmolytic activity in the ileum, its concentration in this organ was also far highest. This was also true for the uptake of demethoxycurcumin into the pulmonary artery and in the aorta where highest concentration was well correlated with the vasodilating activity. Chronotropic activity in the right atrium also

Table 1. Relative uptake of curcumin, demethoxycurcumin, bisdemethoxycurcumin and tetrahydrocurcumin in respective tissue samples. Each data point represents Mean ± SEM of three experiments performed on different tissue samples.

Tissue	Curcumin	Demethoxy-curcumin	Bisdemethoxy-curcumin	Tetrahydro-curcumin
	Mean ± SEM (mg/g)	Mean ± SEM (mg/g)	Mean ± SEM (mg/g)	Mean ± SEM (mg/g)
Ileum	0.79 ± 0.07	0.18 ± 0.04	2.61 ± 0.28	0.23 ± 0.00
Aorta	3.26 ± 0.36	10.56 ± 1.50	10.58 ± 0.01	7.48 ± 0.47
Right Atrium	1.00 ± 0.12	13.96 ± 4.44	2.23 ± 0.25	0.31 ± 0.01
Papillary Muscles	1.93 ± 0.17	17.99 ± 5.05	2.233 ± 1.09	4.82 ± 0.63
Pulmonary Artery	4.13 ± 1.00	35.62 ± 1.83	28.83 ± 5.12	0.94 ± 0.01

correlated with far highest concentrations of demethoxycurcumin in the right atrium while tetrahydrocurcumin with negligible tissue levels showed no activity. A correlation with tissue levels was also true for demethoxycurcumin and tetrahydrocurcumin which both showed the most pronounced inotropic activity. Besides tissue levels we also observed substrate specificity at least for curcumin which selectively showed a positive inotropic effect. The higher uptake of demethoxycurcumin and bisdemethoxycurcumin in different organ preparations might be due to increased stability at physiological pH preventing their degradation to non-active ferulic acid, feruloyl methane and vanillin (41). Also tetrahydrocurcumin is considered much more stable compared to curcumin both in plasma and 0.1M phosphate buffer, irrespective to pH (42).

To summarize, our data showed that demethoxycurcumin and bisdemethoxycurcumin showed more pronounced spasmolytic, vasodilating and negative inotropic activity than curcumin indicating that both curcuminoids significantly contributed to the observed pharmacological effects of *C. longa* extract. Enriched *C. longa* extracts with a higher content of demethoxycurcumin and bisdemethoxycurcumin is therefore highly favorable leading to more therapeutic efficacy. Unfortunately, there are no data in the literature about the bioavailability and pharmacokinetics of demethoxycurcumin, bisdemethoxycurcumin and tetrahydrocurcumin. Further studies are therefore highly warranted to elucidate bioavailability and pharmacokinetics of these compounds in animal models and humans.

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Conflict of interest: None declared.

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