Investigation of the Spasmolytic Activity of the Flavonoid Fraction of *Achillea millefolium* s.l. on Isolated Guinea-pig Ilea

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Summary

The spasmolytic activity of a flavonoid fraction of a commercial sample of yarrow (*Achillea millefolium* s.l.), its main flavonoids as well as quercetin and two flavonoid metabolites were investigated on isolated terminal guinea-pig ilea. The aglycones quercetin, luteolin and apigenin exhibited the highest antispasmodic activities with IC_{50} values of 7.8 µmol/L, 9.8 µmol/L and 12.5 µmol/L, respectively. Rutin and the flavonoid metabolites homoprotocatechuic acid and homovanillic acid showed no significant effects on contractility of the terminal ilea. From the results on the spasmolytic activity of the flavonoid fraction, the glycosides and the respective aglycones it is concluded that in tea prepared from yarrow the concentration of the flavonoids is high enough to exert a spasmolytic effect in the gut, which is mainly caused by blockade of the calcium inward current, but additionally also by mediator-antagonistic effects.

Zusammenfassung

Untersuchung der spasmolytischen Aktivität der Flavonoid-Fraktion aus *Achillea millefolium* s.l. am isolierten Meerschweinchendünndarm

Die Flavonoid-Fraktion einer kommerziell erhältlichen Probe von *Achillea millefolium* s.l. sowie ihre Hauptflavonoide, Quercetin und zwei Flavonoid-Metabolite wurden am isolierten Meerschweinchendünndarm hinsichtlich ihrer spasmolytischen Wirkung getestet. Die Aglykone Quercetin, Luteolin und Apigenin erwiesen sich mit IC_{50}-Werten von 7,8 µmol/L, 9,8 µmol/L und 12,5 µmol/L als am stärksten antispasmodisch wirksam. Rutin und die Flavonoid-Metabolite Homoprotocatechinsäure und Homovanillinsäure veränderten die Kontraktilität der isolierten terminalen Ilea nicht signifikant. Von den Ergebnissen kann abgeleitet werden, dass die Konzentration der im Schafgarbentee enthaltenen Flavonoide hoch genug ist, um zur spasmolytischen Wirkung im Magen-Darm-Trakt beizutragen. Die Wirkung ist hauptsächlich auf einen calciumantagonistischen Effekt und teilweise auf eine Mediator-vermittelte Reduktion der Kontraktilität zurückzuführen.

Key words

- *Achillea millefolium* s.l., flavonoids
- Flavonoids, isolated guinea-pig ileum, spasmolytic activity
- Spasmolytics
- Yarrow

Arzneim.-Forsch./Drug Res. 56, No. 8, 582–588 (2006)

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1. Introduction

Herbal teas from different species of the *Achillea millefolium* group are quite commonly used against gastrointestinal disorders due to the antiphlogistic, spasmolytic and antimicrobial activities [1, 2]. Some investigations to confirm the antispasmodic effects of the drug were already performed, but no detailed information about the responsible substances were described [2, 3, 4]. Flavonoids as some of the main compounds in *Achillea millefolium* L. [1, 2] are known to possess spasmolytic activities besides many other pharmacological effects [5], and these compounds are extracted into teas and tinctures as yarrow is mainly applied.

In this paper the spasmolytic activity of the flavonoids in *A. millefolium* L. was examined. The flavonoid pattern and content in a drug sample (*A. millefolium* s.l., according to the Ph.Eur. 4.00, 2002) was analysed by capillary electrophoresis (CE) in comparison to the amount of flavonoids being extracted into tea preparations. A fraction enriched with flavonoids was prepared by solid phase extraction (SPE) and characterised by CE as well. The antispasmodic effect of this fraction, consisting of flavonoids characteristic for different species of the *A. millefolium* group, was determined on isolated guinea-pig ilea. The single compounds of this fraction were also investigated in this test model. After oral intake flavonoids are, besides other possible pathways, metabolised to their corresponding aglycones and conjugates, and further to phenylacetic or phenylpropionic acids [6–10]. The aglycones apigenin and luteolin occur naturally in the drug. Quercetin as the aglycone of rutin was not described for species of the *A. millefolium* group [11, 12]. As rutin is contained in higher amounts in the drug and due to the deglycosylation of rutin to quercetin [8, 10], this aglycone was also analysed in this study. Furthermore, two flavonoid metabolites (homoprotocatechuic acid and homovanillic acid) were tested for their spasmolytic activity. These substances were detected in urine samples of humans as metabolites after intake of higher amounts of rutin [13]. The investigations should confirm the contribution of the flavonoids of *Achillea millefolium* L. to the antispasmodic effects of the drug due to which yarrow is applied in the treatment of gastrointestinal disorders.

2. Materials and methods

2.1. Analysis of the extracts by CE

The analyses of the flavonoid contents were performed on a SpectraPhoresis 1000 (SpectraPhysics, San Jose, CA, USA) capillary electrophoresis instrument equipped with a high speed scanning multi-wavelength detector. The samples were introduced into an uncoated fused-silica capillary 65.5 cm (58 cm to detector) × 50 µm I.D. (J&W Scientific, Folsom, CA, USA) from its anodic end by hydrodynamic injection for 3 s (1.5 p.s.i.). 25 mmol/L sodium tetraborate (prepared with deionised and distilled water with sodium tetraborate of analytical-reagent grade, supplied by Merck, Darmstadt, Germany) pH 9.3 (adjusted with 1 N NaOH) with 20 % (v/v) methanol added was used as separation buffer. Prior to use the buffer solution was filtered through 0.8 µm membrane filters (0.8 µm cellulose acetate filters, Sartorius, Göttingen, Germany) and degassed. Detection was performed at 270 nm, voltage was maintained at 30 kV, resulting current approximately 28 µA, temperature was kept constant at 35 °C [14]. Identification of the flavonoids was performed by co-electrophoresis with the authentic compounds and comparison of the UV spectra.

Quantification was performed by external standardisation with rutin. A stock solution of 10 mg/10 ml 40 % (v/v) methanol-water was further diluted to obtain 5 standard solutions over the concentration range from 45 to 450 µg/ml. Each day all calibration levels were injected in triplicate for recording the calibration curve, which showed linearity over the selected concentration range and a resulting mean correlation coefficient (R^2) of 0.996. Reproducibility of the method was determined by analysing four standard solutions of rutin (46.3, 138.8, 277.6, 462.7 µg/ml) each in triplicate leading to a mean recovery of 101.59 % (RSD 5.82 %).

For the sample solutions 7 mg of the flavonoid fraction and 200 mg of the lyophilised tea samples were dissolved in 1 ml 40 % (v/v) methanol-water by sonification, centrifuged, filtered and analysed by CE. A 40 % (v/v) methanol-water extract of 1 g of drug was dissolved in 10 ml 40 % (v/v) methanol-water by sonification for 5 min. For all preparations parallel extractions were performed and analysed each in triplicate.

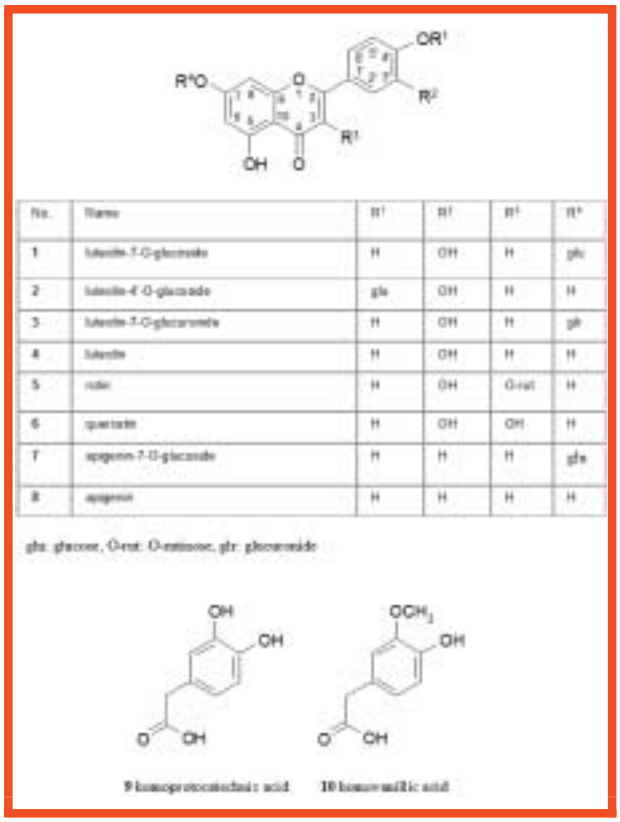
2.2. Test compounds

A flavonoid fraction of *Achillea millefolium* s.l. drug sample [cultivated species, declared as "*Achillea millefolium* L. var. roseo-alba L.", supplied by Aboca, Sansepolcro, Italy, sample 1D5787, cut drug, voucher specimen (Aboca1) is preserved at the Institute of Pharmacognosy, University of Vienna] and its constituents were used to study the effect on contractility of smooth muscle preparations. The mean molecular weight of the flavonoid fraction obtained by SPE (A406080Me) consisting of 2.6 % rutin, 3.7 % apigenin-7-O-glucoside, 2.3 % luteolin-7-O-glucoside, 0.3 % apigenin, 0.5 % luteolin and 0.8 % luteolin-4'-O-glucoside was estimated with 490. The tested constituents were the aglycone luteolin (Extrasynthese, Genay, France; purity ≥ 95 %, MW: 286) and its glycosides luteolin-4'-O-glucoside (Extrasynthese; purity ≥ 95 %, MW: 448) and luteolin-7-O-glucoside (Roth, Karlsruhe, Germany; purity 96.2 %, MG: 448), the aglycone quercetin (Roth; purity ≥ 90 %, MW: 302) and its glycoside rutin (Merck; purity 98.2 %, MW: 610), the aglycone apigenin (Roth; purity ≥ 93 %, MW: 270), and the two flavonoid metabolites 3,4-dihydroxyphenylacetic acid (homoprotocatechuic acid, Fluka, Buchs, Switzerland; purity ≥ 98 %, MW: 168) and 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid, Fluka; purity ≥ 99 %, MW: 182). Structures of the tested compounds are given in Fig. 1. All compounds were soluble in dimethylsulfoxide (DMSO). Stock solutions were prepared with DMSO, and an appropriate amount was added to a 28 ml nutrient solution containing tissue bath in order to obtain final concentrations of 1, 3, 10, 30, and 100 µmol/L of the respective test compound.

The reference compound nifedipine for calcium antagonistic activity, and the agonists acetylcholine, serotonin, phenylephrin and histamine dihydrochloride were purchased from Sigma-Aldrich (Vienna, Austria).

2.3. Preparation of extracts

1 g of powdered drug was extracted under reflux with 100 ml 40 % (v/v) methanol-water for 30 min. After decantation of the solvent, the extraction was repeated twice each with 100 ml 40 % (v/v) methanol-water for 15 min. The combined filtrates were evaporated to dryness under reduced pressure. For quan-



No.	Name	R1	R2	R3	R4
1	luteolin-7-O-glucuronide	H	OH	H	glu
2	luteolin-4′-O-glucoside	glu	OH	H	H
3	luteolin-7-O-glucuronide	H	OH	H	glu
4	luteolin	H	OH	H	H
5	rutin	H	OH	O-rut	H
6	quercetin	H	OH	OH	H
7	apigenin-7-O-glucoside	H	H	H	glu
8	apigenin	H	H	H	H

glu: glucose, O-rut: O-rutinose, glu: glucuronide

9 homoprotocatechuic acid 10 homovanillic acid

Fig. 1: Structures of the tested compounds.

tification of the flavonoids the residue was dissolved in 10 ml 40 % (v/v) methanol-water. For further purification by SPE the residue was dissolved in 10 ml 20 % (v/v) methanol-water and treated in an ultrasonic bath for 5 min. The solution was applied to a C-18 cartridge (Bond Elut, Varian Mega BE-C-18 5 g, 20 ml column), previously conditioned by washing with 2 reservoir volumes (RV) methanol and 2 RV deionised water. After purging with air for 5 min, the cartridge was eluted at a flow rate of 4 ml/min with 4 RV water, 4 RV 20 % (v/v) methanol-water, 2 RV 40 % (v/v) methanol-water, 2 RV 60 % (v/v) methanol-water and 2 RV 80 % (v/v) methanol-water. The fractions eluted with 40, 60 and 80 % methanol were combined containing the main flavonoids. The methanol was removed by evaporation under reduced pressure and the remaining aqueous solution was lyophilised.

Teas of the cut drug were prepared each with 3.5 g cut drug and 150 ml water according to the monographs infus and decoct of the Austrian Pharmacopoeia 2003. Each preparation was prepared twice giving drug to extract ratios of 5:1 for infus and 4.6:1 for decoct.

2.4. Smooth muscle preparation

The effect of the compounds was studied in isolated terminal ilea of the guinea-pig (Institute for Laboratory Animal Science and Genetics, Medical University of Vienna, Himberg, Austria). The animals (either sex, 320–430 g) were kept in automatically controlled temperature conditions (23 ± 2 °C) in 12 h dark-light cycles, with food and water ad libitum. 24 h prior to the experiment, they were deprived of food. The guinea-pigs were killed by a blow on the neck. After quick removal the intestine was cleaned by flushing with nutrient solution, and was placed in oxygenated nutrient solution. The terminal ileum was cut into 2–3 cm long pieces, and a small silver hook was attached to the preparation to allow mounting in the experimental set-up.

2.5. Nutrient solution

The preparations were isolated and stored at room temperature in Krebs-Henseleit Solution with the following composition (in mmol/L): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.05, NaH_2CO_3 24.0, NaH_2PO_4 0.43, glucose 11.0. During the experiments the preparations were superfused with oxygenated (95 % O_2–5 % CO_2) Krebs-Henseleit solution at a temperature of 37 ± 1 °C to guarantee sufficient oxygen supply and appropriate pH of 7.2–7.4.

2.6. Experimental set-up

Isometric measurement of contraction force of KCl (60 mmol/L)-precontracted terminal ilea was performed with a force transducer (Type Fort 10 with a Transbridge™ 4-Channel Transducer Amplifier; World Precision Instruments, Sarasota, FL, USA). A resting tension of 4.9 mN was applied to obtain maximum contractility, and was kept constant throughout the experiments. Signals were recorded continuously with a chart recorder (BD 112 Dual Channel; Kipp&Zonen, Delft, Netherlands).

2.7. Experimental protocol

A resting tension of 4.9 mN was applied to the isolated terminal ileum in order to obtain maximum contractility of the preparation, and was kept constant throughout the experiments. Following an equilibrium period of half an hour the terminal ilea were precontracted with 60 mmol/L KCl containing Krebs-Henseleit Solution. After 10–15 min a new constant level of contraction force was reached, which was followed by a control period of another 15 min. The test compounds were added cumulatively to the bathing solution in rising concentrations every 30 min when a steady-state effect has been reached. The IC_{50} values were estimated graphically.

Studying the discussed calcium antagonistic effect, in a series of experiments the extracellular calcium chloride concentration was increased stepwisely up to a concentration of 10 mmol/L or until the decrease of contractility by the test compound was antagonized.

For measurement of contractions evoked by agonists, isolated terminal ilea were contracted with phenylephrine (0.1, 0.3, 1.0, 3.0 and 10.0 µmol/L), serotonin (0.01, 0.1, 0.3, 1.0, 3.0, 10 and 30 µmol/L), histamine dihydrochloride (0.01, 0.03, 0.1, 0.3, 1.0 and 3.0 µmol/L) and acetylcholine (0.01, 0.03, 0.1, 0.3, 1.0 and 3.0 µmol/L). After maximum contraction was observed at a distinct concentration of the injected agonist the preparation was washed three times before the agonist was applied at the next higher concentration. The same procedure was then performed in presence of the test compound.

Because of insolubility of the compounds in aqueous nutrient solution, an appropriate amount of DMSO had to be used. Therefore, a series of experiments had to be performed using the solvent containing bathing solution without any test compound present.

2.8. Statistics

For statistical analysis the arithmetic means and standard error of the mean (SEM) of n experiments were calculated. “n” gives the number of experiments. The terminal ileum of an animal was cut into 2–3 cm long pieces, which yielded two preparations. Each preparation was used for only one experiment. For each series of experiments at least 3 experiments were performed. If data scattered, the number of experiments was increased.

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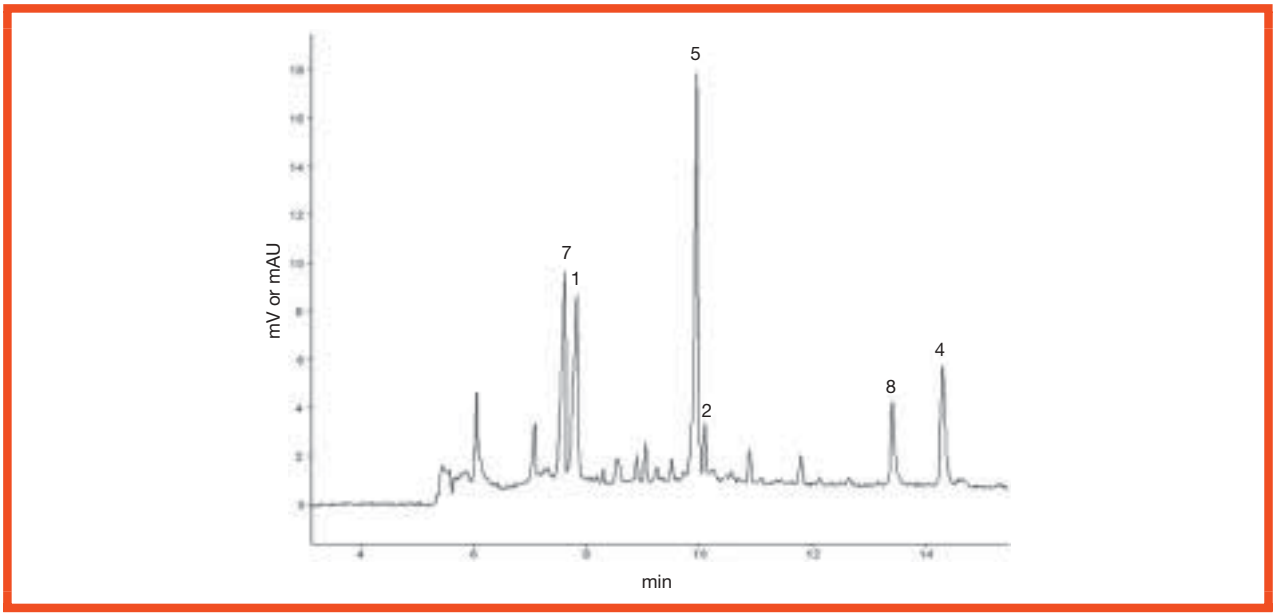

Fig. 2: Electropherogram of the fraction A406080Me (obtained by SPE). Running buffer: 25 mmol/L sodium tetraborate containing 20 % (v/v) of methanol (pH 9.3); capillary: 65.5 cm × 50 µm I.D.; separation voltage: 30kV; column temperature: 35 °C; UV detection: 270 nm.

Statistical significance of the results was evaluated by the Student's t-test for paired observations.

3. Results

3.1. Characterisation of the extracts

Quantification of the flavonoids in a 40 % (v/v) methanol-water extract of the drug by CE showed a total content of 0.91 % flavonoids calculated as rutin. Compounds **5, 1** and **7** were the main compounds, each with 0.12 %, besides **3, 2** and **4** with 0.11, 0.10 and 0.10 %, respectively. Furthermore the amount of flavonoids contained in tea preparations was determined by analysing prepared infusa and decocts. The flavonoid contents were 0.30 and 0.35 % for the infus and the decoct. In both tea preparations, compound **5** was the main compound with 0.10 % (infus) and 0.09 % (decoct) besides **3** (0.07/0.07 %), **1** (0.03/0.05 %), **7** (0.03/0.03 %) and **2** (0.03/0.02 %). Minor amounts of the aglyca **4** and **8** below 0.03 % were also detected.

For investigations of the muscle relaxing activity of the flavonoids in the drug a fraction enriched of flavonoids was prepared by SPE and analysed by CE (Fig. 2). The quantification of the flavonoid content in this fraction resulted in 10.2 %. Concentrations of 2.6, 3.7 and 2.3 % were determined for **5, 7** and **1.** The aglycones **4** and **8** occurred in 0.3 and 0.5 %, **2** in 2.6 %. All the main compounds of the drug occurred in the fraction except **3.** This component eluted from the C-18 cartridge with 20 % (v/v) methanol-water together with phenolic acids in the drug.

3.2. Spasmolytic activity in terminal ileum

At the concentrations used (0.5 to 100 µl/28 ml) the solvent DMSO decreased force of contraction concentration-dependently. These concentrations of DMSO correspond to the amount of solvent in the presence of the test compounds at concentrations between 1 and 100 µmol/L. Up to 3.5 µl/28 ml DMSO, which is the solvent concentration at 10 µmol/L test compound, no effect on contractility was observed. At 10 and 35 µl DMSO in 28 ml nutrient solution (which corresponds to the amount present with 30 and 100 µmol/L test compound) force of contraction was decreased by 4.4 % and 10 %, respectively (n = 6). Therefore, results obtained with the flavonoid fraction and its constituents were corrected for this solvent induced decrease in contractility.

The tested flavonoid fraction of *Achillea* concentration-dependently decreased the force of contraction in isolated terminal ilea. At a mean flavonoid concentration of 30 µmol/L the decrease was 42.6 ± 2.2 % (IC_{50} = 54.5 ± 4.3 µmol/L) (n = 4) (Fig. 3 and 4). Thus we tested which of the components contributed to the spasmolytic activity of the flavonoid fraction.

After deglycosylation of a flavonoid in the enterocyte or luminal hydrolysis the released aglycone is absorbed by passive diffusion [8]. Therefore, we compared the effect of glycosides with the respective aglycone. The force of contraction was not significantly changed by **5** (n = 3) within the tested concentration range of 1 to 100 µmol/L, while its aglycone **6** reduced the force of contraction concentration-dependently and significantly at concentrations higher than 1 µmol/L with an IC_{50} of 7.8 ± 3.1 µmol/L (n = 3) (Fig. 3).

In vivo quercetin derivatives are deconjugated and converted to hydroxyphenylacetic acids [9, 10]. Thus the effect of the main metabolites **9** (n = 3) and **10** (n = 3) was studied as well. As illustrated in Fig. 3, these metabolites did not show any effect on the contractility of terminal ilea.

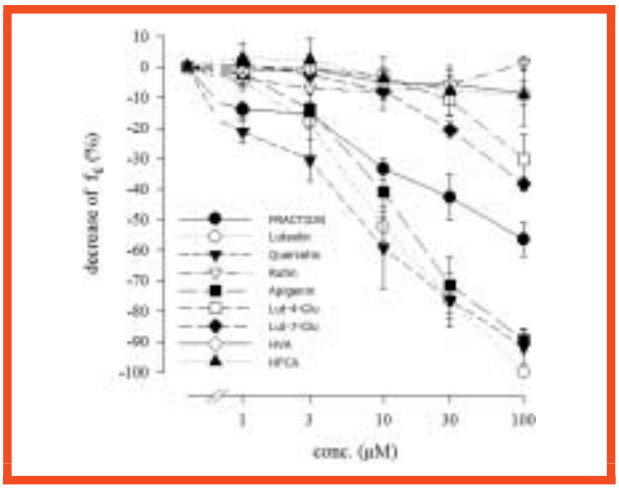

Fig. 3: Concentration-response curves are shown for the flavonoid fraction (n = 4), the aglycones 4 (Luteolin, n = 4), 6 (Quercetin, n = 5) and 8 (Apigenin, n = 3), the glycosides 5 (Rutin, n = 3), 2 (Lut-4-Glu, n = 4) and 1 (Lut-7-Glu, n = 3) as well as for the two metabolites 9 (HVA, n = 3) and 10 (HPCA, n = 3).

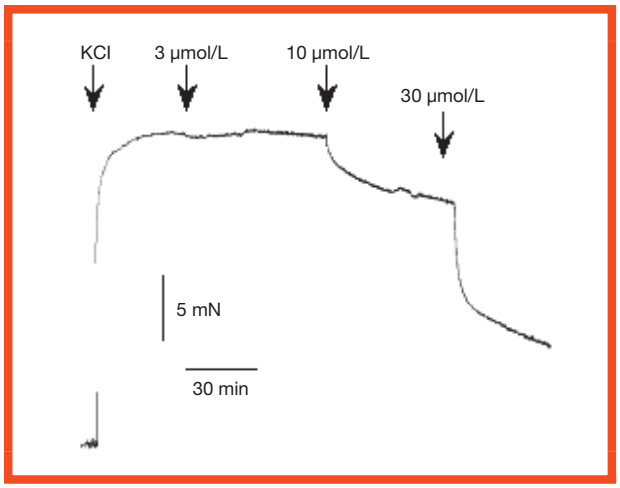

Fig. 4: Original recordings of contractility measurements in isolated terminal ileum of the guinea pig. Increasing the potassium concentration for precontraction of the preparation is indicated with an arrow as well as the cumulative addition of rising concentrations of the flavonoid fraction.

The two glycosides **1** and **2** decreased the contraction force of precontracted terminal ilea concentration-dependently (Fig. 3) with an IC_{50} of 245 ± 18 (n = 3) and 305 ± 35 µmol/L (n = 4), respectively. Again, the aglycone was much more potent than the respective glycosides (Fig. 3): **4** reduced the force of contraction with an IC_{50} of 9.8 ± 1.9 µmol/L (n = 4). The aglycone **8** showed a similar spasmolytic activity with an IC_{50} of 12.5 ± 2.1 µmol/L (n = 3) (Fig. 3).

3.3. Calcium antagonistic effect

To verify the assumption that the blockade of the calcium inward current is responsible for the spasmolytic activity of the flavonoid fraction and some of its constituents, a series of experiments was performed, increasing the extracellular calcium concentration stepwisely in order to antagonize the decrease of contraction force.

The spasmolytic effect of the flavonoid fraction at a concentration of 3 µmol/L was antagonized by 6.4 mmol/L $CaCl_2$(n = 3), but the decrease of f_c by the 10 µmol/L fraction (n = 4) could not be opposed by increasing the calcium chloride concentration (Fig. 5). The antagonistic effect of calcium ions to the fraction induced decrease of f_c was mainly due to the components **4** (10 µmol/L: n = 4, 30 µmol/L: n = 4) and **6** (10 µmol/L: n = 3, 30 µmol/L: n = 3), and to a lesser extent to **8** (10 µmol/L: n = 3). The spasmolytic effect of 10 and 30 µmol/L **4** was antagonized by 3.7 and 3.9 mmol/L $CaCl_2$, respectively, and that of 10 and 30 µmol/L **6** by 4.9 and 5.7 mmol/L $CaCl_2$, respectively. The decrease of f_c by 10 µmol/L **8** was abolished by 8.6 mmol/L $CaCl_2$. The effect of 30 µmol/L **8** was not antagonized by $CaCl_2$ concentrations up to 10 mmol/L (n = 3).

3.4. Effect on contractions generated by mediators

The effect on contractions induced by agonists such as serotonin, phenylephrine, acetylcholine and histamine dihydrochloride was tested.

Serotonin (0.01–30 µmol/L) evoked contractions were not significantly affected by the flavonoid fraction (30 µmol/L, n = 5), components **4** (10 µmol/L, n = 3) and **8** (30 µmol/L, n = 3), but were non-significantly reduced by **6** (30 µmol/L, n = 3). Contraction amplitudes induced by acetylcholine (0.01–3 µmol/L) were not changed significantly by the flavonoid fraction (30 µmol/L, n = 6) and **4** (10 µmol/L, n = 3), but were re-

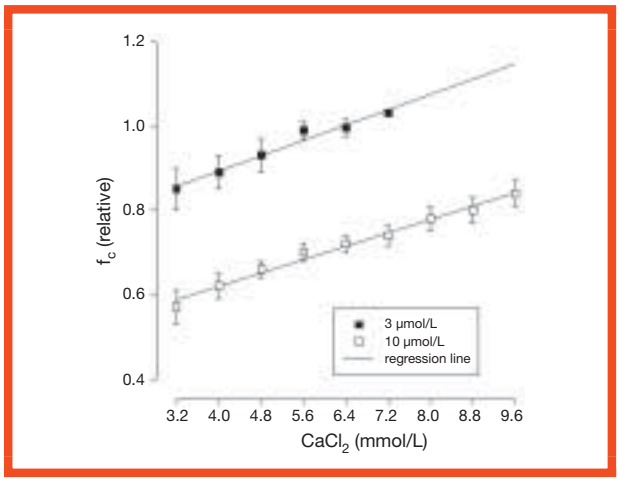

Fig. 5: The reduction of f_c by 3 (n = 3) and 10 µmol/L (n = 4) flavonoid fraction is attenuated by increasing the extracellular $CaCl_2$ concentration. The regression lines were calculated to estimate the calcium concentration needed for complete abolition of the reduction of f_c. The effect of 3 µmol/L flavonoid fraction is antagonized by 6.4 mmol/L $CaCl_2$, while the effect of the higher concentration could not be abolished completely. Mean values with SEM are given. The control is indicated as 1 on the ordinate.

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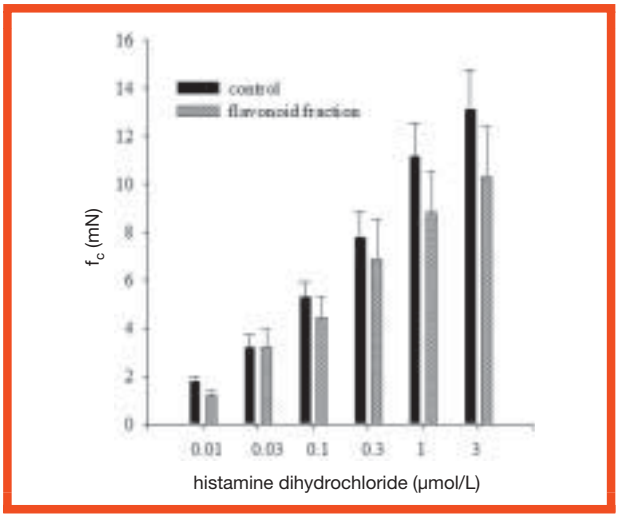

Fig. 6: The contraction amplitude in mN is plotted against the added histamine hydrochloride concentration in µmol/L. Dark columns represent control values, hatched columns the response in the presence of 30 µmol/L flavonoid fraction (n = 5).

duced by **6** (30 µmol/L, n = 3) and **8** (30 µmol/L, n = 3). Phenylephrine (0.1–10 µmol/L) evoked contractions were neither affectd by the flavonoid fraction (30 µmol/L, n = 4) nor by the spasmolytic components **4** (10 µmol/L, n = 3), **6** (30 µmol/L, n = 3) and **8** (30 µmol/L, n = 3). Contractions caused by histamine dihydrochloride (0.01–3 µmol/L) were non-significantly decreased by **6** (30 µmol/L, n = 3) and **8** (30 µmol/L, n = 3). The reduction of f_c by the flavonoid fraction (30 µmol/L, n = 5) and **4** (10 µmol/L, n = 3), however, was significant ($P < 0.05$) (Fig. 6).

4. Discussion

The experiments revealed the spasmolytic activity of the *Achillea* flavonoid fraction on intestine smooth muscle. Thus we studied which constituent of the flavonoid fraction was responsible for this effect and compared the effect of the contained glycosides with the respective aglycones and the metabolites.

The spasmolytic effect of the aglycones **4** and **8** on guinea-pig ileum is comparable with the vasodilating effect reported for rat aorta [15, 16], while Lin et al. [17] found only a ten times weaker activity for **4** in rat thoracic aorta.

The force of contraction was not significantly changed by the glycoside **5** within the tested concentration range, while its aglycone **6** reduced the force of contraction concentration-dependently and significantly. Similarily the aglycone **4** was much more potent than the respective glycosides **1** and **2.** These data, showing reduced biological activity of flavonoid glycosides, confirm the results found in vascular smooth muscle [18] and isolated rat ileum [19]. Furthermore, the glycosides **1** and **2,** which possess the same aglycone, decreased the contraction force of precontracted terminal ilea concentration-dependently to almost the same extent, indicating that the position of the sugar moiety does not influence the spasmolytic activity.

In vivo quercetin derivatives are deconjugated and converted to hydroxyphenylacetic acids [9, 10]. Thus the effect of the main metabolites **9** and **10** was studied as well. These did not contribute to the spasmolytic activity, since they did not show any effect on contractility of the terminal ilea.

In the next series of experiments the mechanism of the antispasmodic action of yarrow was studied. Therefore the isolated preparations were precontracted by high potassium- or agonist-containing bathing solution. Contraction with potassium-rich nutrient solution is caused by depolarization of the membrane and thereby activation of L-type calcium channels. The reduction of the force of contraction in such precontracted preparations thus gives evidence for the blockade of the calcium inward current by the test compound. The effect of calcium antagonistic drugs like diltiazem can be abolished in this way [20] as was done with the reference compound nifedipine. The spasmolytic effect of 3 µmol/L flavonoid fraction was antagonized by $CaCl_2$, but the decrease of f_c by 10 µmol/L fraction could not be opposed by increasing the calcium chloride concentration. The antagonistic effect of calcium ions on the fraction induced decrease of f_c was mainly due to the components **4** and **6,** and to a lesser extent to **8.** However, the mechanism of the spasmolytic effect of **6** is discussed controversially [18, 21, 22, 23]. It was reported that the antagonistic molar ratio of diltiazem to the calcium ion was constant and thus the results were interpreted as competitive antagonism [20]. With the tested flavonoid fraction and the contained flavonoids, however, we never found a constant molar ratio, which means that other mechanisms contribute to the decrease of the contraction force. Nonetheless, the results suggest a spasmolytic activity of **4, 6** and **8** mainly by suppressing the calcium inward current as previously shown for compound **8** in guinea-pig ileum [23] and the other flavonoids in vascular smooth muscle [24], although other mechanisms may contribute to the spasmolytic activity [25].

Thus isolated terminal ilea were stimulated with agonists in the absence and presence of the flavonoids. Compound **6** caused a serotonin antagonistic effect as was also seen in rat aorta, although in contrast to guinea-pig terminal ileum, this effect was found to be significant in vascular smooth muscle [26]. This difference in activity might be due to the numerous 5-HT (serotonin) receptor subtypes in various tissues.

Acetylcholine induced contraction amplitudes were not affected by the flavonoid fraction, but were only reduced by **6** and **8,** which confirms the published data for **6** [27, 28, 29], although the decrease that we found with **6** was not significant. Thus the weak anticholinergic effect of components **6** and **8** is obviously not reflected in an anticholinergic activity of the flavonoid fraction.

Phenylephrine evoked contractions were neither affected by the flavonoid fraction nor by the spasmolytic

aglycones **4, 6** and **8.** This result obtained in intestinal smooth muscle is in contrast to the findings for the aglycones **6** and **8** on rat aorta [25, 30], which report a decrease in phenylephrine induced contractility.

Histamine induced contractions were non-significantly decreased by **6** and **8,** which confirms published data [28, 31]; although these authors reported a significant decrease of contractility by those compounds. The reduction of f_c by the flavonoid fraction and **4,** however, was significant. It was reported that compound **4** can block the release of histamine from mast cells [32].

From the calculated IC_{50} values for the spasmolytic activity of the flavonoid fraction, the glycosides and the respective aglycones it is concluded that in tea prepared from yarrow the concentration of the flavonoids is high enough to exert a spasmolytic effect in the gut, which is mainly caused by blockade of the calcium inward current, but additionally also by mediator-antagonistic effects.

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