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Inhibition of Human cAMP-Phosphodiesterase as a Mechanism of the Spasmolytic Effect of *Matricaria recutita* L.

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Mechanisms underlying the spasmolytic activity of chamomile still remain unclear. Inhibition of cAMP- and cGMP-phosphodiesterases (PDE) is one of the mechanisms operated by spasmolytic drugs. In this study, the effect of chamomile on PDE was investigated. Human platelet cAMP-PDE and recombinant PDE5A1 were assayed in the presence of infusions prepared from sifted flowers and capitula. LC-ESI-MS/MS analysis showed different compositions in infusions made with sifted flowers and capitula. Chamomile inhibited cAMP-PDE activity (IC_{50} = 17.9−40.5 μg/mL), while cGMP-PDE5 was less affected (−15% at 50 μg/mL). Among the individual compounds tested, only flavonoids showed an inhibitory effect (IC_{50} = 1.3−14.9 μM), contributing to around 39% of the infusion inhibition; other compounds responsible for cAMP-PDE inhibition still remain unknown. Although experimental evidence supporting the use of chamomile for gastrointestinal minor spasms dates back to the fifties, cAMP-PDE inhibition as a likely mechanism underlying the spasmolytic activity is reported for the first time.

KEYWORDS: Chamomile; *Matricaria recutita* L.; phosphodiesterase; spasmolytic activity; infusion

INTRODUCTION

Matricaria recutita L. (synonymous with *Matricaria chamomilla*, chamomile) is an annual herbaceous plant indigenous to Europe and western Asia but is commonly used also in Australia and the United States. The consumption of chamomile in the form of tea is rated to be more than 1 million cups per day. Over 120 constituents have been identified in chamomile flowers, including polysaccharides and fatty acids, terpenoids [α-bisabolol (BI) and its oxides], and the coumarins herniarin (HE) and umbelliferone (UM) (*1*). Other major constituents of the flowers include several phenolic compounds, primarily the glycosides of apigenin (AP), quercetin (QU), patuletin (PA), luteolin (LU), and acetyl and malonyl glycosides (*2–4*). Large amounts of phenolic acids including caffeic (CA), chlorogenic acids (CHA), and ferulic acid (FA) derivatives have also been identified (*2, 5*). Flavonoids and phenolic acids are soluble in hot water, and the amounts uptaken from frequent consumption of infusions or teas are not negligible (*2, 6*).

Chamomile is used as an herbal remedy for its reputation as a carminative, antispasmodic, mild sedative, anti-inflammatory, and antiseptic (for a review, see ref *7*). The German Commission E approved chamomile for internal use to treat gastrointestinal (GI) spasms and inflammatory diseases of the GI tract (*8*). Methods of administration are either the hydroalcoholic extract or the tea of dried flowers, as reported in ESCOP Monographs. The spasmolytic activity of water extracts has been documented in vitro on the isolated guinea pig ileum (*9*), and some individual constituents of chamomile extract, such as BI, HE, and the flavonoids PA, AP, and their monoglycoside derivatives, have shown spasmolytic activity as well (*9–11*). Smooth muscle relaxant properties were also attributed to the *cis*-spiroether (*12*). Although the spasmolytic activity of chamomile preparations is widely acknowledged, the mechanisms underlying smooth muscle relaxation remain unclear.

The cyclic nucleotides cAMP and cGMP regulate the GI smooth muscle tone causing relaxation. Inhibition of phosphodiesterases (PDEs), which catalyze the hydrolysis of cAMP and cGMP to 5′-AMP and 5′-GMP (*13*), is one of the mechanisms operated by spasmolytic drugs (*14*). PDE3A and PDE5A isoforms are the main cAMP- and cGMP-specific PDEs occurring in smooth muscle cells, respectively. PDE3A is inhibited by cGMP, thus providing a feedback control on cAMP levels.

This study was undertaken to investigate whether the spasmolytic effect of the *M. recutita* L. infusions could be ascribed to PDE inhibition. While PDE inhibition by flavonoids has already been described (*15–20*), the effect of chamomile infusion on cGMP- and cAMP-PDE was never investigated. Another aspect considered in this study is that for use as a tea, chamomile is available as an unpacked drug or packed in sachets containing

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Table 1. Recovery and Concentration of Infusions Prepared with Capitula (CFI) and Sifted Flowers (SFI)[a]

chamomile sample	recovery % vs dried drug weight (mean ± SD)	infusion concentration (mg/mL) (mean ± SD)
CFI 1	28.5 ± 2.2	4.8 ± 0.4
CFI 2	28.2 ± 1.4	3.9 ± 0.2
SFI 1	27.7 ± 2.3	4.1 ± 0.3
SFI 2	23.6 ± 1.2	3.9 ± 0.2
SFI 3	27.4 ± 2.6	4.6 ± 0.4
SFI 4	21.7 ± 1.6	3.6 ± 0.3

[a] The infusion concentration was calculated on the basis of the dry residue obtained after lyophilization. Results are the mean ± SD of six samples obtained from two different batches.

either the entire dried flowers (capitula) or sifted flowers, consisting of the tubular part of the flower only. Therefore, the activity of cAMP- and cGMP-PDEs has been investigated in the presence of infusions prepared from both types of chamomile since the distribution of the constituents in the different parts of the flower is not homogeneous (*2*). Infusions were analyzed by liquid chromatography−electrospray ionization−tandem mass spectrometry (LC-ESI-MS/MS) to verify whether compositional differences affected biological activity.

MATERIALS AND METHODS

Reagents. Culture medium Dulbecco's modified Eagle's medium, trypsin, protease inhibitors, and all chemical reagents for cell culture were purchased from Sigma Aldrich (Milan, Italy). Penicillin, streptomycin, and L-glutamine were from Gibco (Grand Island, NY). Fetal calf serum was provided by Mascia Brunelli SpA (Milan, Italy). COS-7 cell line was purchased from American Type Culture Collection (ATCC) (Manassas, VA). Superfect reagent for transient transfections was obtained from Qiagen GmbH (Hilden, Germany). The expression plasmid pcDNA3 containing the full-length cDNA of phosphodiesterase-5A1 (PDE5A1) was a kind gift of Prof. C. S. Lin (Department of Urology, University of California, San Francisco, CA). [^{3}H]cGMP and [^{3}H]cAMP were from Amersham Pharmacia Biotech (Amersham Place, Little Chalf-ont, Buckinghamshire, United Kingdom). DEAE-Sephadex A25 was from Pharmacia (Uppsala, Sweden). cGMP, cAMP, AMP, *Crotalus adamanteus* snake venom, aminophylline, and phloridzin were purchased from Sigma Aldrich. Sildenafil, AP, LU, apigenin-7-O-glucoside (AP-7-O-glu), luteolin-7-O-glucoside (LU-7-O-glu), rutin (RU), hyperoside (HYP), CA, FA, and chlorogenic acid (CGA) were provided by Sequoia Research Products (Oxford, United Kingdom); patuletin-7-O-glucoside (PA-7-O-glu) was from Apin Chemicals Ltd. Quinic acid (QA), BI, HE, UM, and chamazulene (CHAM) were from Extrasynthèse (Lyon, France). All compounds were of high-performance liquid chromatography purity grade.

Preparation of the Chamomile Infusions and Percent Recovery. Different brands of chamomile were purchased from drugstores: two brands with sachets containing capitula (CFI 1 and CFI 2) and four brands with sachets containing sifted flowers (SFI 1−4). For each brand, six sachets were chosen randomly, and the contents of each sachet were infused for 10 min with deionized water at boiling temperature (2 g/120 mL). The solution was then filtered and lyophilized, and the recovery (% w/w) was calculated (**Table 1**). The infusions were combined and used for LC-MS/MS analyses and for the biological activity.

Validation of the Analytical Method and Analysis of CFI and SFI by LC-MS/MS. Qualitative and quantitative analyses were carried out by LC-MS/MS using a LC system P4000 (Thermo Separation Products) coupled with an LCQ ion-trap mass spectrometer (Thermo Finnigan, United States) equipped with an ESI source operating in the negative mode. The column used was a Reprosil-PUR C18-AQ 50 mm × 2 mm, 3 μm (Dr. Maisch GmbH, Ammerbuch, Germany), with a flow rate of 0.3 mL/min. A gradient elution was performed using water acidified with 0.1% formic acid (A) and methanol (B). The gradient program for qualitative analyses was as follows: 0−3 min, 90% (A); 3−5 min, from 90 to 70% (A); 5−25 min, from 70 to 50% (A); 25−40 min, from 50 to 0% (A); 40−41 min, 0% (A); 41−42 min, from 0 to 90% (A); 42−52 min, 90% (A). The gradient program for quantitative analyses of AP-7-O-glu and LU-7-O-glu was as follows: 0−3 min, 95% (A); 3−25 min, from 95 to 40% (A); 25−26 min, from 40 to 95% (A); 26−45 min, 95% (A). To optimize the separation of PA-7-O-glu and HYP for their quantification, the gradient was modified as follows: 0−3 min, 95% (A); 3−30 min, from 95 to 40% (A); 30−40 min, from 40 to 30% (A); 40−41 min, from 30 to 95% (A); 41−51 min, 95% (A). The operating conditions for MS analysis were as follows: spray voltage, 4 kV; capillary temperature and voltage, 250 °C and −28 V, respectively; sheath gas and auxiliary gas flow, 75 and 25 arbitrary units, respectively; and tube lens offset, 10 V. The collision energy was set at 35% of 5 V. AP-7-O-glu, LU-7-O-glu, PA-7-O-glu, and HYP were identified by comparison of the retention times and mass spectra with those of authentic compounds. Quantification of the compounds under study was performed by monitoring specific ions derived from the collision of the $[M - H]^-$ ion of each compound (**Table 2**). Calibration curves were prepared with phloridzin (1 μg/sample), as internal standard, and increasing amounts of the authentic phenols (25−500 ng). Linearity of the calibration curves was assessed by R^2 (**Tables 3** and **4**). Intraday (**Table 3**) and interday (**Table 4**) precision [coefficient of variation (CV) %] and accuracy [error %, calculated as (obtained value − true value)/true value × 100] of the analytical method were calculated over a series of blank samples spiked with different amounts of the authentic compounds estimated on the basis of the calibration curves.

Table 2. Precursor and Product Ions Selected for ESI-MS/MS Analysis[a]

compound	precursor ion (*m*/*z*) $[M - H]^-$	product ion (*m*/*z*)
AP-7-O-glu	431	269
LU-7-O-glu	447	285
PA-7-O-glu	493	331
HYP	463	301

[a] Quantification was performed by monitoring ions derived from the collision of the $[M - H]^-$ ion of each compound. One specific product ion was selected for each compound.

Table 3. Intraday Precision (CV %) and Accuracy (Error %) of the LC-MS/MS Method[a]

compound	ng added	ng found	CV %	error %	R^2
PA-7-O-glu	100	99.6	6.3	−0.4	
	250	250.6	3.6	0.2	0.998
	500	499.6	0.8	−0.1	
LU-7-O-glu	50	46.8	1.8	−6.4	
	100	104.2	1.1	4.2	0.998
	250	248.9	0.1	−0.4	
AP-7-O-glu	100	97.5	1.4	−2.5	
	250	254.0	0.9	1.6	0.999
	500	498.5	0.1	−0.3	
HYP	25	23.2	1.2	−7.2	
	50	53.0	3.3	6.0	0.997
	100	97.4	2.7	−2.6	

[a] Values are the means of three replications/day. Linearity for calibration curves was assessed by R^2.

Platelet Homogenate Preparation and Assay for cAMP-PDE Activity. Platelet homogenate was prepared from the blood of healthy volunteers as previously described (*20*); briefly, the blood fraction enriched in platelets, obtained from the blood of healthy volunteers, was submitted to two centrifugations at 160*g* for 10 min at room temperature. The pellet was removed, and platelet-rich plasma (PRP) was centrifuged at 1000*g* for 15 min. The resulting pellet was resuspended in 10 mM Tris/HCl, pH 7.4 (2/5 of the initial volume). The suspension was centrifuged at 1000*g* for 15 min, and the pellet was suspended in the Tris/HCl buffer, pH 7.4 (1/12 of the initial volume). All of these steps were performed at 4 °C. Cells were disrupted by freezing and thawing for three times obtaining the homogenate (*21*), and cell lysates were stored at −80 °C. The total protein concentration was measured according to Bradford (*22*).

Table 4. Interday Precision (CV %) and Accuracy (Error %) of the LC-MS/MS Method[a]

compound	ng added	ng found	CV %	error %	R^2
PA-7-O-glu	100	96.3	3.5	−3.7	
	250	255.7	1.9	2.3	0.998
	500	497.7	0.4	−0.5	
LU-7-O-glu	50	47.5	2.9	−5.0	
	100	101.5	2.6	1.5	0.999
	250	248.6	0.5	−0.6	
AP-7-O-glu	100	97.8	0.5	−2.2	
	250	253.5	0.3	1.4	0.999
	500	498.7	0.06	−0.3	
HYP	25	24.0	5.4	−4.0	
	50	51.3	3.3	2.6	0.998
	100	98.9	1.5	−1.1	

[a] Values are the means of three days replications. Linearity for calibration curves was assessed by R^2.

The cAMP-PDE activity was determined according to the method of Kincaid and Manganiello (*23*) with minor modifications (*20*). Platelet lysate (64 μg of protein/mL) was incubated with 0.5 μM cAMP and 63 nCi [^{3}H]cAMP suspended in 30 mM Tris-HCl, pH 7.4, and 4 mM $MgCl_2$; the final reaction volume was 250 μL. After 5 min of incubation at 30 °C, the reaction was stopped with 0.1 N HCl. Samples were then incubated for a further 4 min at 70 °C with 5 mM AMP and 5 mM cAMP, and the pH was adjusted to 7 on ice with 0.1 N NaOH. Samples were then incubated for 20 min at 37 °C with 50 μL of nucleotidase from *Crotalus adamanteus* snake venom (1 mg/mL in 0.1 M Tris-HCl, pH 8.0) to cleave AMP to the corresponding nucleoside. The reaction was stopped, and the nucleoside formed during the incubation was separated from the unreacted substrate by DEAE-Sephadex A25 column chromatography. The eluted [^{3}H]adenosine was counted in a β-scintillation counter. cAMP-PDE activity was expressed as pmol of product formed/min/mg of protein. Results are expressed as pmol of product formed/min/mg of protein. SFI and CFI were tested at 5−300 μg/mL and individual compounds at 10 μM. Inhibition (%) by 100 μM aminophylline, used as a reference compound, was 74.5 ± 1.3 [mean ± standard deviation (SD), $n = 11$].

Expression and Enzyme Assay of Human Recombinant PDE5A1. Human recombinant PDE5A1 was prepared by expression of the full-length cDNA of PDE5A1 into COS-7 cells as previously described (*24*). The PDE5A1 activity was determined according to the method of Kincaid and Manganiello (*23*) with minor modifications (*25*). CFI and SFI were tested at 50 μg/mL. The PDE-5 inhibitor sildenafil, used as a reference compound, showed 70% inhibition at 100 nM.

Statistical Analysis. A two-way analysis of variance was used to test for statistical differences. Differences with $p < 0.05$ were considered to be significant. IC_{50} values were calculated from the sygmoidal curves using Graph Pad Prism 4, and each point represents the mean of nine replications ± the SD.

RESULTS

Recovery and Analysis of CFI and SFI. As shown in **Table 1**, the recovery from the infusions of sifted flowers and capitula was similar, representing around 30% calculated on the dried drug weight. **Figure 1** shows the qualitative profiles of CFI (**A**) and SFI (**B**). Flavonoids HYP (**1**), PA-7-O-glu (**2**), LU-7-O-glu (**3**), and AP-7-O-glu (**4**) were identified and quantified. The corresponding aglycones were not found. The concentration of these flavonoids in CFIs and SFIs is reported in **Figure 2**. CFIs contained more AP-7-O-glu (0.26 and 0.32% w/v) than LU-7-O-glu (0.10 and 0.13% w/v), whereas the opposite was observed for SFIs (0.02−0.09 and 0.12−0.20% for AP-7-O-glu and LU-7-O-glu, respectively). PA-7-O-glu was the most abundant (0.37−0.80%, w/v) in all of the infusions. The HYP concentration was very low in all infusions and ranged between 0.02 and 0.06%.

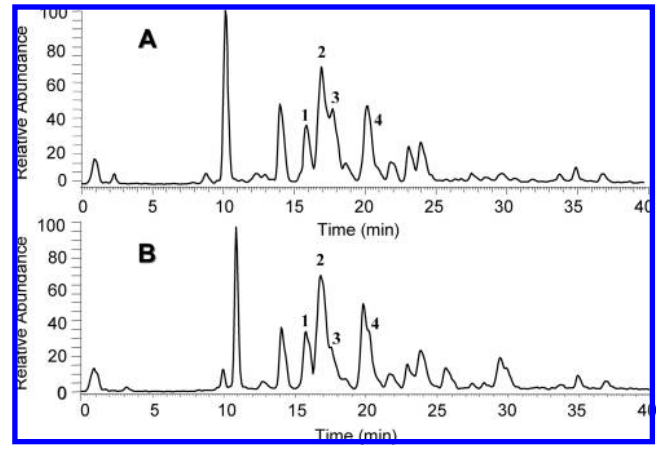

Figure 1. Total ion current profile of chamomile infusions from capitula (**A**) and sifted (**B**) flowers. HYP (**1**), patuletin-7-O-glu (**2**), luteolin-7-O-glu (**3**), and apigenin-7-O-glu (**4**).

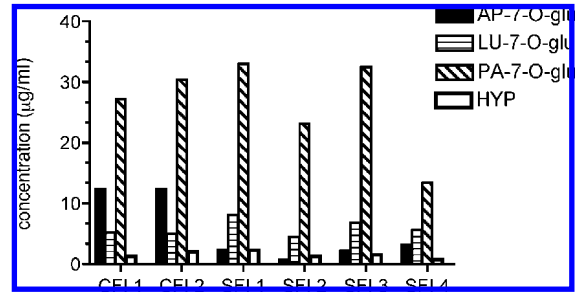

Figure 2. Levels of flavonoids in chamomile infusions from capitula (CFI) and sifted flowers (SFI). Quantitative determination of the individual compounds AP-7-O-glu, LU-7-O-glu, PA-7-O-glu, and HYP was carried out by LC-MS/MS in ESI negative mode. Results are the means of at least two injections.

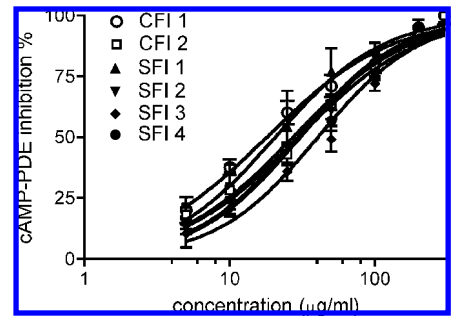

Figure 3. Effect of chamomile infusions from capitula (CFI) and sifted (SFI) flowers on cAMP-PDE. The enzyme activity was determined as described in the Materials and Methods. Results are the means ± SD of three experiments performed in triplicate.

Effect of SFI, CFI, and the Individual Compounds on PDE Activities. Control cAMP-PDE and PDE5A1 activities were 76.9 ± 9.5 (mean ± SD, $n = 14$) and 56.3 ± 9.4 pmol × min^{-1} × mg protein^{-1} (mean ± SD, $n = 8$), respectively. CFI and SFI exhibited a concentration-dependent inhibition of cAMP-PDE (**Figure 3**); the IC_{50} of the two CFIs was 17.9 and 27.2 μg/mL, whereas the IC_{50} for SFIs ranged from 20.5 to 40.5 μg/mL (**Table 5**). A weak, still statistically significant inhibition of PDE5A1 by CFIs and SFIs (7−14% vs control) was observed but at a concentration as high as 50 μg/mL.

Our interest was then to identify the active principles of chamomile responsible for the effect. At this regard, we tested the identified compounds AP-7-O-glu, LU-7-O-glu, PA-7-O-glu, HYP, and other phenols, RU, CA, FA, and CHA that were

Table 5. Effect of Chamomile Infusions and Individual Flavonoids on cAMP-PDE Activity

chamomile infusions	cAMP-PDE IC_{50} (μg/mL ± SD)
CFI 1	17.9 ± 2.1
CFI 2	27.2 ± 3.5
SFI 1	20.6 ± 1.8
SFI 2	29.9 ± 2.9
SFI 3	40.5 ± 6.7
SFI 4	29.8 ± 4.6

compounds	cAMP-PDE IC_{50} (μM ± SD)
AP-7-O-glu	10.2 ± 1.7
LU-7-O-glu	14.9 ± 2.6
PA-7-O-glu	14.9 ± 0.4
HYP	11.8 ± 0.9
AP	4.1 ± 0.9
LU	1.3 ± 0.1

supposed to be present in the water extracts. Because intestinal metabolism to the corresponding aglycones via β-glycosidase activity is thought to increase the biological activity of flavonoids, thus AP and LU were also tested for cAMP-PDE activity.

The investigation was also extended to other constituents of chamomile (i.e., BI, HE, UM, and CHAM), which are known to be present in hydroalcoholic extracts. At 10 μM, only flavonoids inhibited cAMP-PDE, while the other compounds were inactive (**Figure 4**). For the compounds active at 10 μM, a concentration response inhibition was investigated and reported in **Figure 5**. The IC_{50} values ranged from 1.3 to 14.9 μM, and the potency order was as follows: LU > AP > AP-7-O-glu = PA-7-O-glu = HYP = LU-7-O-glu (**Table 5**). To verify the potential synergy between components, the effect of the individual compounds and of the reconstituted mixture was investigated and compared to the effect of the infusion CFI 1. In this experiment, the concentration of the flavonoids in the assay was that expected to be present in 300 μg/mL of the infusion according to the quantitative analysis. The results are shown in **Figure 6**. The effect of the reconstituted mixture is the summation of the single effects, thus excluding a synergistic effect. The inhibition by the flavonoid mixture was around 39%, roughly half of the infusion inhibition. This result means that other constituents still unidentified contribute to the overall effect of the infusion.

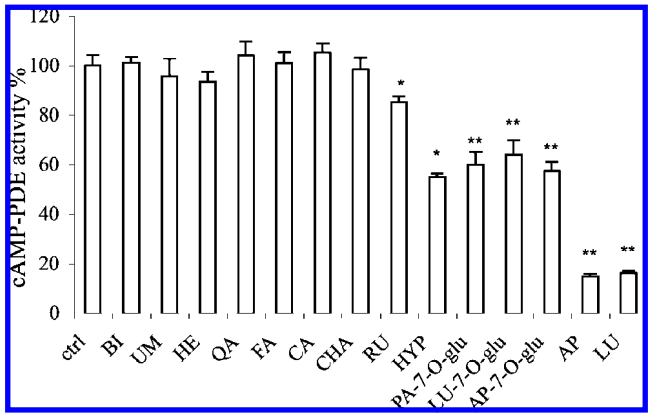

Figure 4. Effect of individual compounds (10 μM) occurring in chamomile on cAMP-PDE. cAMP-PDE activity was determined as reported in the Materials and Methods. Results are the means ± SD of three experiments performed in triplicate. *$p \leq 0.0005$; **$p \leq 0.0001$.

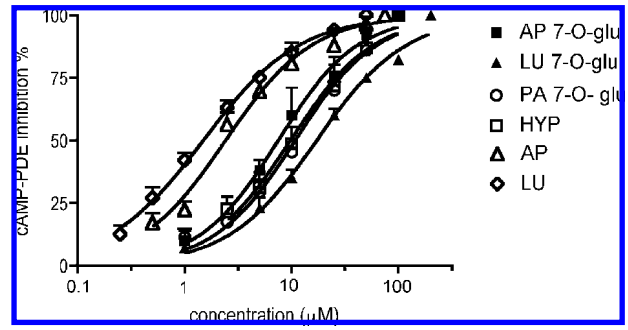

Figure 5. Effect of flavonoids on human platelet cAMP-PDE. cAMP-PDE activity was determined according to the Materials and Methods. Results are the means ± SD of three experiments performed in triplicate.

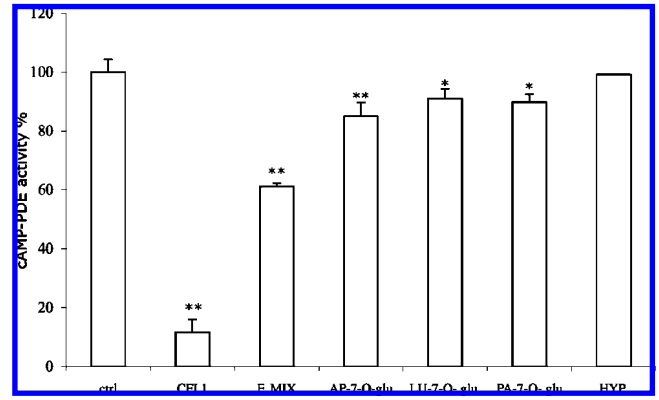

Figure 6. Effect of the infusion CFI 1 (300 μg/mL) in comparison with the reconstituted flavonoid mixture [F. MIX, AP-7-O-glu (1.8 μM), LU-7-O-glu (0.75 μM), PA-7-O-glu (3.5 μM), and HYP (0.18 μM)] and the individual compounds AP-7-O-glu (1.8 μM), LU-7-O-glu (0.75 μM), PA-7-O-glu (3.5 μM), and HYP (0.18 μM). The concentration of the flavonoids in the assay was that expected to be present in 300 μg/mL of the infusion according to the quantitative analysis. *$p < 0.05$; **$p < 0.01$.

DISCUSSION

The objective of the present study was to verify the hypothesis that cAMP- and cGMP-PDE could be one of the targets for the spasmolytic effect of chamomile. Because chamomile is sold in two packaging forms containing either the entire capitula or the sifted flowers, we were interested to verify whether the different packaging modes would influence the biological effect.

Whether a tea was prepared with sachets containing capitula or sifted flowers, the concentration of the extracts was not statistically different, but the composition was greatly affected; the AP-7-O-glu/LU-7-O-glu ratio was 3 in CFIs and 0.3 in SFIs. The nonhomogenous distribution of AP-7-O-glu in the different parts of the flowers (*26*) can explain the different quantitative profile. Capitula consist of both ligulate (where AP-7-O-glu is particularly abundant) and tubular flowers, whereas only tubular flowers make sifted chamomile.

Our results show that chamomile inhibits cAMP-PDE activity, while cGMP-PDE5 seems to be less affected. These data support the hypothesis that smooth muscle relaxation caused by chamomile is mediated via the decrease of cAMP degradation rather than that of cGMP. The variability of the results from a chamomile brand to the other does not allow one to draw clear-cut conclusions whether capitula and sifted flowers affect the enzyme activity differently.

Among the individual compounds tested on cAMP-PDE activity, only flavonoids showed an inhibitory effect, contributing to around 39% of the infusion inhibition, while the phenolic acids, BI, and coumarins were inactive. However, other ingredients of chamomile infusion responsible for cAMP-PDE inhibition still remain unknown. Previous studies have demonstrated that the biological activity exerted by flavonoids is highly dependent on the presence or absence of the glycoside residue and that the position and nature of the sugar residue may modify the uptake of the compounds by the small intestine (*27*). Intestinal metabolism of flavonoid glucosides leading to the corresponding aglycones is thought to increase the effects of flavonoids. As expected, AP and LU showed an inhibitory effect higher than their 7-O-glucosides, thus suggesting that in vivo PDE inhibition, and consequently smooth muscle relaxation, may be strengthened by the presence of aglycones. Similar results were also reported by Ko et al. (*17*), in particular for LU and LU-7-O-glu. Furthermore, previous in vivo experiments have already shown that the flavonoid fraction of other herbal infusions and particularly the aglycones quercetin, LU, and AP exhibit high antispasmodic activity on isolated guinea pig ilea (*28*).

Because the potency of the individual flavonoid glucosides was comparable, the contribution of each compound to the overall in vitro effect depends on the concentration of each in the infusion. The HYP concentration is very low and therefore insufficient to produce any inhibition. It has to be underlined that chamomile infusions contain a variety of other AP derivatives, such as the mono- and diacetate, malonyl, and caffeoyl esters (*2*–*4*), which could not be assayed due to current unavailability, but may act as well.

In conclusion, chamomile tea has been widely consumed as a pleasant drink or as a medicinal remedy for centuries. Although experimental evidence supporting its use for GI minor spasms dates back to the fifties, this is the first study reporting cAMP-PDE inhibition by chamomile infusions as a likely mechanism underlying the spasmolytic activity. However, other mechanisms may also be concurrent.

ABBREVIATIONS USED

PDE5A1, phosphodiesterase-5A1; ATCC, American Type Culture Collection; GI, gastrointestinal; AP-7-O-glu, apigenin-7-O-glucoside; LU-7-O-glu, luteolin-7-O-glucoside; PA-7-O-glu, patuletin-7-O-glucoside; HYP, hyperoside; CHAM, chamazulene; QA, quinic acid; HE, herniarin; UM, umbelliferone; AP, apigenin; LU, luteolin; RU, rutin; CA, caffeic acid; CHA, chlorogenic acid; FA, ferulic acid; BI, bisabolol; CFI, infusion from capitula flowers; SFI, infusion from sifted flowers; CV, coefficient of variation.

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