Inhibitory Effects of Hesperetin Derivatives on Guinea Pig Phosphodiesterases and Their Ratios Between High- and Low-Affinity Rolipram Binding

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ABSTRACT: The phosphodiesterase (PDE)4 molecule exists as two distinct conformers, $PDE4_H$ and $PDE4_L$, which have high and low affinities, respectively, for the selective PDE4 inhibitor, rolipram. The inhibition of $PDE4_H$ and $PDE4_L$ is associated with adverse responses, such as nausea, vomiting, and gastric hypersecretion, and with anti-inflammatory and bronchodilator effects, respectively. We determined the therapeutic ($PDE4_H/PDE4_L$) ratios of hesperetin-7-*O*-methylether, hesperetin-5,7,3′-*O*-trimethylether (HTME), hesperetin-7-*O*-acetate, hesperetin-7,3′-*O*-diacetate, hesperetin-5,7,3′-*O*-triacetate (HTA), hesperetin-5,7,3′-*O*-tripropionate, hesperetin-5,7,3′-*O*-tributyrate, hesperetin-5,7,3′-*O*-triisobutyrate, and hesperetin-5,7,3′-*O*-tripivatate, and compared these ratios to those of hesperetin, hesperetin-7,3′-*O*-dimethylether, hesperidin, and hesperidin-3′-*O*-methylether to identify derivatives with therapeutic ratios and to characterize the structure–activity relationships among these compounds. The activities of PDE isozymes 1 through 5 were measured using a two-step procedure using [^{3}H]adenosine 3′,5′-cyclic monophosphate or [^{3}H]guanosine 3′,5′-cyclic monophosphate as substrates. The inhibitory concentration (IC_{50}) for 50% of PDE4 inhibition and effective concentration (EC_{50}) for replacing 50% of [^{3}H]rolipram binding on high-affinity rolipram-binding sites was taken as the $PDE4_L$ and $PDE4_H$ value, respectively. The HTME and the HTA dually inhibited PDE3 and PDE4, and displayed $PDE4_H/PDE4_L$ ratios of 18.3 and 20.8, respectively, suggesting that they may be candidate drugs for treating asthma and chronic obstructive pulmonary disease (COPD) because the combined inhibition of PDE3 and PDE4 has synergistically anti-inflammatory and bronchodilator effects in COPD patients. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:2120–2127, 2013

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INTRODUCTION

Phosphodiesterases (PDEs) have been classified into at least 11 distinct enzyme families that hydrolyze adenosine 3′,5′ cyclic monophosphate (cAMP) and/or guanosine 3′,5′ cyclic monophosphate (cGMP).[1] PDE isozymes are classified as calcium/calmodulin-dependent (PDE1), cGMP-stimulated (PDE2), cGMP-inhibited (PDE3), cAMP-specific (PDE4), or cGMP-specific (PDE5), and are found in many tissues.[2] Recent increasing evidence suggests that selective inhibitors of these PDEs may have clinical applications. The selective PDE5 inhibitors, sildenafil, tadalafil, and vardenafil, are used to treat erectile dysfunction.[3] In addition, selective inhibitors of PDE3, such as milrinone, vesnarinone, and enoximone, may produce beneficial effects in the treatment of chronic congestive heart failure. Although the morbidity and mortality of patients with severe chronic heart failure increased with long-term oral use of milrinone,

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short-term clinical use of the compound has been approved for the treatment of acute decompensated heart failure.[3]

The PDE4 isozyme plays important roles in airway smooth muscle, pulmonary nerves, many immune cells, and proinflammatory responses that are relevant to the pathogenesis of asthma. Thus, selective PDE4 inhibitors are being investigated for treating asthma.[3] Rolipram, an archetypal inhibitor of PDE4, may be beneficial for treating asthma; however, nausea and vomiting were reported in clinical trials.[4] The PDE4 isozyme exists as two distinct conformers, $PDE4_H$ and $PDE4_L$, which have high and low affinities for rolipram, respectively. It is generally believed that the inhibition of $PDE4_H$ and $PDE4_L$ is associated with an adverse response, such as nausea, vomiting, and gastrohypersecretion, and with anti-inflammatory and bronchodilating effects, respectively,[3] providing a rational basis for the design of new synthetic compounds with high $PDE4_H/PDE4_L$ ratios. We previously found quercetin and ayanin to have the potential for treating asthma and chronic obstructive pulmonary disease (COPD), displaying $PDE4_H/PDE4_L$ ratios of greater than 30 and 19, respectively.[5] However, quercetin has been reported to inhibit PDE3 and PDE4 at micromolar concentrations and PDE1 and PDE2 at submolar concentrations.[6] The inhibitory effects of quercetin on PDE isozymes 1 through 4 are nonselective; thus, it does not represent an ideal lead compound for the synthesis of derivatives to generate new therapeutic agents for asthma and COPD.

Hesperetin (5,7,3′-trihydroxy-4′-methoxyflavanone), one of the most-common flavonoids in *Citrus* species, is present in many herbal medicines as glycosides. Hesperidin and neohesperidin are abundant in the peel of *Citrus aurantium* L. (Rutaceae), a traditional Chinese medicine (TCM) that is used as an expectorant and a stomach tonic, as well as in the treatment of capillary fragility and hypertension.[7] These glycosides are easily hydrolyzed by glycosidase to form hesperetin after ingestion. Men with higher hesperetin intake have lower mortality from cerebrovascular disease and lung cancer, and lower incidences of asthma.[8] In addition, hesperetin has been reported to selectively inhibit PDE4 activity[6] and to have anti-inflammatory effects in mice.[9] Therefore, hesperetin may represent a suitable target to prepare its structure analogs as potential therapeutic agents for the treatment of asthma and COPD. Leaving the flavanone skeleton intact, the peripheral hydroxyl groups of the hesperetin was modified by *O*-alkylation and *O*-esterization to increase lipid solubility. In our present study, we determined the inhibitory effects of various hesperetin derivatives on the activities of PDE isozymes 1 through 5, and found hesperetin-5,7,3′-*O*-trimethylether (HTME) and hesperetin-5,7,3′-*O*-triacetate (HTA) dually inhibited PDE3/4 activities. We also compared the $PDE4_H/PDE4_L$ ratios for these derivatives, including HTME and HTA, to those of hesperetin, hesperidin, hesperetin-7,3′-*O*-dimethylether (HDME), and hesperidin-3′-*O*-methylether (HDM) to identify those with therapeutic ratios, and to characterize the structure–activity relationships among the structural analogs.

MATERIALS AND METHODS

Drugs and Reagents

The hesperetin-7-*O*-methylether (HME) and HDME were synthesized from hesperetin, as previously described.[10] The HTME (mol. wt., 344.28 g/mol)[11] and hesperetin-7-*O*-acetate (HA)[12] were also synthesized according to previously described methods. Hesperetin-7,3′-*O*-diacetate (HDA), HTA (mol. wt., 428.27 g/mol), hesperetin-5,7,3′-*O*-tripropionate (HTPP), hesperetin-5,7,3′-*O*-tributyrate (HTB), hesperetin-5,7,3′-*O*-triisobutyrate (HTIB), and hesperetin-5,7,3′-*O*-tripivatate (HTP) were synthesized according to other previously described methods.[13] The hesperetin derivatives were identified using mass, nuclear magnetic resonance, ultraviolet and infrared spectroscopic methods. The purities of all the compounds exceeded 98% as assessed by high-performance liquid chromatograph. Hesperetin and hesperidin were purchased from Sigma–Aldrich Chemical (St. Louis, Missouri). The HDM was purchased from the Tokyo Chemical Industry (Tokyo, Japan). The structures of the derivatives are shown in Figure 1. All experimental protocols used in our study were approved by the Animal Care and Use Committee of Taipei Medical University.

PDE Inhibitions

The activities of PDE isozymes 1 through 5 in guinea pig lung and heart homogenates[14] were measured using a two-step procedure according to a previously described method,[15] using [^{3}H]cAMP or [^{3}H]cGMP. We determined the inhibitory effects of the hesperetin derivatives and the reference drugs, vinpocetine,[16] *erythro*-9-(2-hydroxy-3-nonyl) adenine (EHNA),[17] milrinone,[18] Ro 20-1724,[19] and zaprinast,[20] respectively. The value of IC_{50} was defined as the concentration of the hesperetin derivative or reference drug that inhibited 50% of the total PDE isozyme activity.

Determination of $PDE4_H$ Values

The $PDE4_H$ values were determined according to previously described methods.[21,22] The EC_{50} value, the concentration of the hesperetin derivative or