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Ginsenosides Are Potent and Selective Inhibitors of Some Calmodulin-Dependent Phosphodiesterase Isozymes†

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ABSTRACT: The effects of various ginsenosides on calmodulin-dependent phosphodiesterase isozymes have been investigated. Ginsenosides were found to be potent inhibitors of bovine heart calmodulin-dependent phosphodiesterase and the 60-kDa isozyme of bovine brain calmodulin-dependent phosphodiesterase but not of the 63-kDa isozyme of bovine brain calmodulin-dependent phosphodiesterase. Since the inhibition of phosphodiesterase by ginsenosides was overcome by increasing the concentration of calmodulin, this suggests that ginsenosides act specifically and reversibly against the action of the calmodulin. These compounds therefore should be valuable tools to investigate the diverse physiological roles of distinct phosphodiesterase isozymes.

Calmodulin-dependent cyclic nucleotide phosphodiesterase (3′,5′-cyclic-nucleotide 5′-nucleotidohydrolase, EC 3.1.4.17) is one of the key enzymes involved in the complex interaction between the cyclic nucleotide and Ca^{2+} second-messenger systems. The activity of calmodulin-dependent cyclic nucleotide phosphodiesterase (CaMPDE)[1] was found to be widely distributed in mammalian tissues and other eukaryotes [for reviews, see Beavo et al. (1982), Sharma et al. (1988), Beavo (1990), and Wang et al. (1990)]. The enzyme has been purified close to homogeneity from both bovine brain (Morrill et al., 1979; Sharma et al., 1980; Kincaid & Vaughan, 1983; Kincaid et al., 1984; Shenolikar et al., 1985) and bovine heart (LaPorte et al., 1979; Hansen & Beavo, 1982; Sharma, 1991) and has been extensively characterized. Recent studies showed that CaMPDE exists in different isozymic forms which exhibit distinct molecular and/or catalytic properties (Hansen & Beavo, 1982; Sharma et al., 1984; Purvis et al., 1981; Vandermeers et al., 1983; Sharma & Wang, 1986; Rossi et al., 1988; Sharma, 1991). Bovine brain 60-kDa phosphodiesterase isozyme (BB60kDaCaMPDE), bovine heart CaMPDE (BHCaMPDE), and bovine lung CaMPDE isozymes are almost identical in terms of immunological properties; however, they are differentially regulated by calmodulin (Mutus et al., 1985; Hansen & Beavo, 1986; Sharma & Wang, 1986; Sharma, 1991). The BHCaMPDE, BB60kDaCaMPDE, and BB63kDaCaMPDE are differentially regulated by cAMP-dependent protein kinase and calmodulin-dependent protein kinase (Sharma, 1991; Sharma & Wang, 1985, 1986).

Ginseng (*Panax quinquefolium*) is used for treatment of heart failure and to protect tissues from damage when an organism is in stress (Wagner & Liu, 1987). Among the actions studied are CNS depressant, antipsychotic, anticonvulsant, and antifatigue actions (Shibata et al., 1985). Therefore, in this study, we examined possible effects of ginseng root on bovine brain and heart CaMPDE isozymes. We found that the BHCaMPDE and BB60kDaCaMPDE isozymes were inhibited by ginseng root and various ginsenosides, but BB63kDaCaMPDE isozyme was not.

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[1] Abbreviations: CaM, calmodulin; CaMPDE, calmodulin-dependent cyclic nucleotide phosphodiesterase; BHCaMPDE, bovine heart calmodulin-dependent cyclic nucleotide phosphodiesterase; BB60kDaCaMPDE, bovine brain 60-kDa calmodulin-dependent cyclic nucleotide phosphodiesterase; BB63kDaCaMPDE, bovine brain 63-kDa calmodulin-dependent cyclic nucleotide phosphodiesterase.

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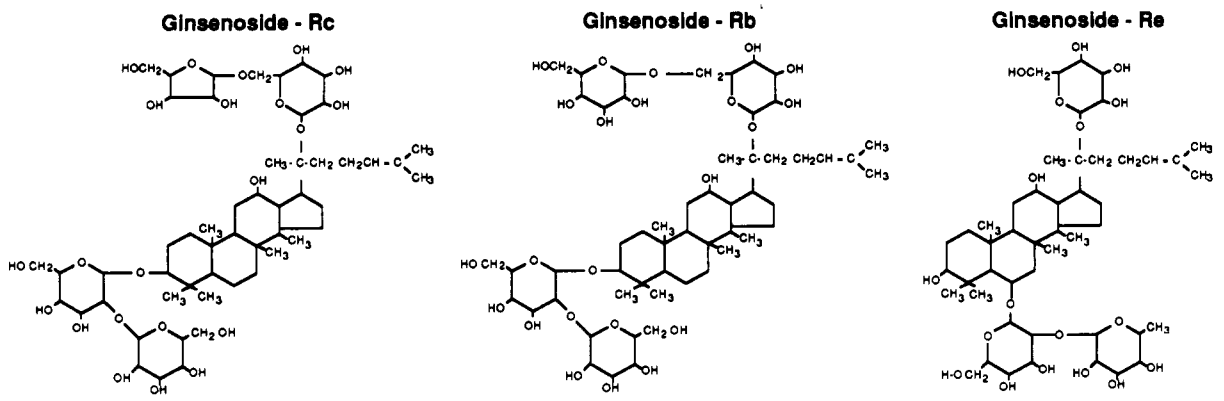

FIGURE 1: Structure of various ginsenosides.

EXPERIMENTAL PROCEDURES

Materials

BHCaMPDE and BB60kDaCaMPDE and BB63kDaCaMPDE isozymes were purified as described (Sharma, 1991; Sharma et al., 1985). Bovine brain calmodulin was purified as described by Sharma (1990). Ginseng root and ginsenosides (Re, Rb, and Rc) were purchased from Sigma. The chemical structures of various ginsenosides are shown in Figure 1. General laboratory reagents were of analytical grade or better.

Methods

Phosphodiesterase Assay. CaMPDE activity was measured as described (Sharma & Wang, 1979). The reaction mixture contained 40 mM Tris-HCl, 40 mM imidazole, 5 mM magnesium acetate, pH 7.5, 0.5 unit of 5-nucleotidase, 1.2 mM cAMP, and other components as described in the figure legends in a total volume of 0.9 mL. Reactions were carried out at 30 °C for 30 min. One unit of PDE is defined as the amount of enzyme which, when fully activated, hydrolyzes 1 μmol of cAMP/min at 30 °C.

Preparation of Crude Extract from Ginseng Roots. Ginseng roots were crushed and suspended in glass double-distilled water. The suspension was incubated for 10 min in a boiling water bath and then cooled on ice. The cooled sample was filtered through cheesecloth and then Whatman No. 1 filter paper. The light yellow extract was dried by lyophilization and stored over anhydrous Na_2SO_4 in a desiccator.

RESULTS

Inhibitory Effect of Ginseng Root Extract on Various Calmodulin-Dependent Phosphodiesterase Isozymes. The effect of ginseng root extract on BHCaMPDE and BB60kDaCaMPDE and BB63kDaCaMPDE isozymes was examined. Figure 2 shows that a crude extract of ginseng roots caused a concentration-dependent inhibition of BHCaMPDE and the BB60kDaCaMPDE isozyme but not of the BB63kDaCaMPDE isozyme. The IC_{50} at 60 ng/mL calmodulin was 25 μg/mL. Increasing the concentration of calmodulin resulted in a rightward shift of the inhibition curve (data not shown). This suggests that a component of the crude extract competes with the ability of calmodulin to activate the enzyme. The inhibitory effect of the crude extract was directed specifically toward the calmodulin-activated activity of the enzyme. When the enzyme was treated briefly with trypsin to eliminate its response to calmodulin, the crude extract had no effect on the enzyme activity. Furthermore, the basal activity of the native enzyme was not inhibited (data not shown). These results demonstrate that ginseng root extracts

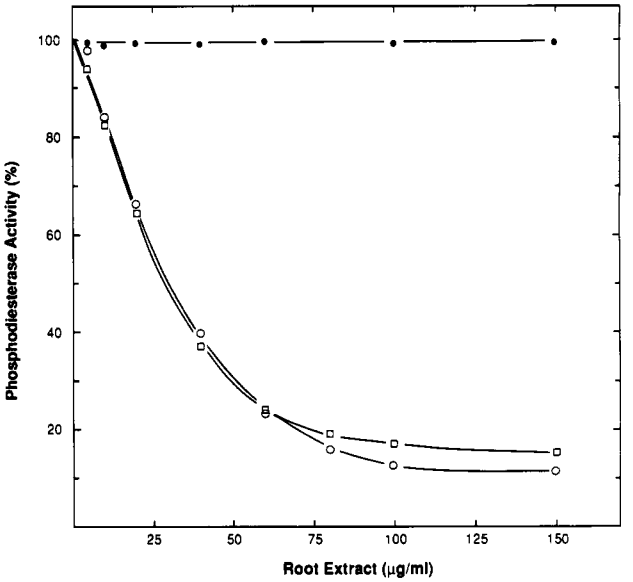

FIGURE 2: Effect of ginseng root extract on calmodulin-dependent phosphodiesterase isozymes. CaMPDE activity was determined as described under Experimental Procedures. Purified BB63kDaCaMPDE (●), BB60kDaCaMPDE isozyme (○), and BHCaMPDE (□) were assayed with various concentrations of ginseng root extract in the presence of 60 ng/mL calmodulin.

inhibit BHCaMPDE and the BB60kDaCaMPDE isozyme but not the BB63kDaCaMPDE isozyme.

Inhibition of Calmodulin-Dependent Phosphodiesterase Isozymes by Ginsenosides. To determine the identify of the active component of ginseng roots, the effects of purified ginsenosides on the various CaMPDE isozymes were examined. BHCaMPDE and the BB60kDaCaMPDE isozyme were inhibited by various ginsenosides, but the BB63kDaCaMPDE isozyme was not (Figure 3).

The IC_{50} values for the inhibition are summarized in Table I. These results suggest that BHCaMPDE and the BB60kDaCaMPDE isozyme have similar affinities for the ginsenosides.

Reversal of Calmodulin-Dependent Phosphodiesterase Isozyme Inhibition by Calmodulin. The possibility that the inhibition of CaMPDE isozymes by ginseng root extract and ginsenosides is the result of competition between CaMPDE and ginsenosides for calmodulin was examined. The high concentrations of the calmodulin can reverse the inhibition of BHCaMPDE and BB60kDaCaMPDE by the ginsenosides. Figure 4 shows the effect of the ginsenoside Rc on the dose–response curve of the activation of BHCaMPDE by the calmodulin. While the ginsenoside Rc markedly inhibited BHCaMPDE reaction at low concentrations of the calmodulin, the enzyme inhibition was largely abolished by high concen-

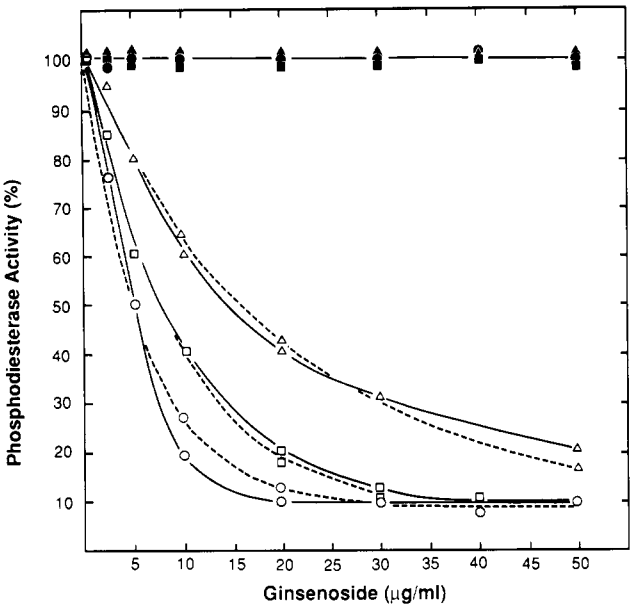

FIGURE 3: Effect of various ginsenosides on bovine brain and heart calmodulin-dependent phosphodiesterase. CaMPDE activity was determined as described under Experimental Procedures. Purified BB63kDaCaMPDE isozyme with ginsenoside Rc (●—●), Rb (■—■), and Re (▲—▲); BB60kDaCaMPDE isozyme with Rc (○—○), Rb (□—□), and Re (△—△); and BHCaMPDE with Rc (○- - -○), Rb (□- - -□), and Re (△- - -△) were assayed in the presence of 60 ng/mL calmodulin.

Table I: Effect of Various Ginsenosides on Calmodulin-Dependent Phosphodiesterase Isozymes[a]

	IC_{50} (μg/mL) for ginsenoside		
CaMPDE isozyme	Rc	Rb	Re
BB60kDCaMPDE	4.0	7.0	12.0
BHCaMPDE	4.0	7.5	14.0

[a] CaMPDE isozyme activities were determined as described under Experimental Procedures at 60 ng/mL calmodulin in the presence of various ginsenosides. The IC_{50} value is defined as the concentration of ginsenoside required to produce 50% inhibition of CaMPDE activity. IC_{50} values were calculated graphically as shown in Figure 3. Each value is the mean of two experiments.

trations of the calmodulin (Figure 4). When higher concentrations of the ginsenoside Rc were used, higher amounts of the calmodulin were required to overcome the enzyme inhibition (results not shown). To further test the reversibility of CaMPDE inhibition by the ginsenoside Rc, the effect of a high concentration of the calmodulin on the progress curve of an inhibited BHCaMPDE reaction was examined. Figure 5 shows that the inhibition of BHCaMPDE activity by ginsenoside Rc could be completely reversed by the addition of excess calmodulin (10 μg/mL). Similar results were also obtained when BB60kDaCaMPDE isozyme was used (results not shown). These results further suggest that the ginsenosides are specific and reflect a simple competition for available calmodulin.

DISCUSSION

A variety of pharmacological agents have been reported to inhibit CaMPDE, and this occurs mostly via Ca^{2+}-dependent association with the protein. We have demonstrated that BHCaMPDE and the BB60kDaCaMPDE isozyme, but not the BB63kDaCaMPDE isozyme, are inhibited by ginsenoside. The inhibition of CaMPDE isozymes was overcome by addition of excess calmodulin, suggesting that ginsenosides may be calmodulin antagonists. The BB63kDaCaMPDE isozyme is

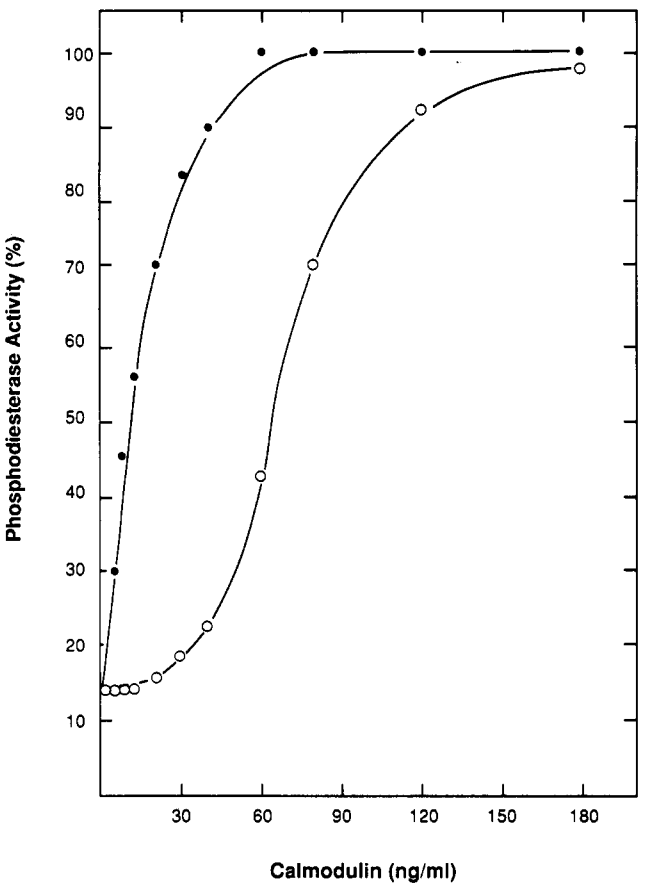

FIGURE 4: Effect of calmodulin on the inhibition of calmodulin-dependent phosphodiesterase by the ginsenoside Rc. CaMPDE reaction with 0.04 unit/mL BHCaMPDE in the absence (●) and presence (○) of 5 μg/mL ginsenoside Rc was carried out at various concentrations of the calmodulin.

kinetically different from the BB60kDaCaMPDE isozyme, BHCaMPDE, and bovine lung isozyme (Wu et al., 1992). Although BB60kDaCaMPDE isozyme, BHCaMPDE, and bovine lung CaMPDE isozyme are almost identical in terms of immunological and kinetic properties (Sharma & Wang, 1986; Sharma, 1991; Wu et al., 1992), they are differentially activated by calmodulin (Mutus et al., 1985; Hansen & Beavo, 1986; Sharma & Wang, 1986; Sharma, 1991). The BHCaMPDE isozyme has significantly higher affinity for calmodulin than the BBCaMPDE isozymes (Mutus et al., 1985; Hansen & Beavo, 1986; Sharma & Wang, 1986; Sharma, 1991). The bovine lung CaMPDE isozyme has the highest affinity for calmodulin, since it contains calmodulin as a subunit (Sharma & Wang, 1986). Similarly, the porcine brain CaMPDE has been shown to have a lower affinity for calmodulin than the isozyme from porcine artery (Keravis et al., 1987). These differences in the affinities for calmodulin of isozymes from different tissues may have physiological significance during $[Ca^{2+}]$ transients. Ginsenosides may be useful tools to elucidate the roles of specific CaMPDE isozymes.

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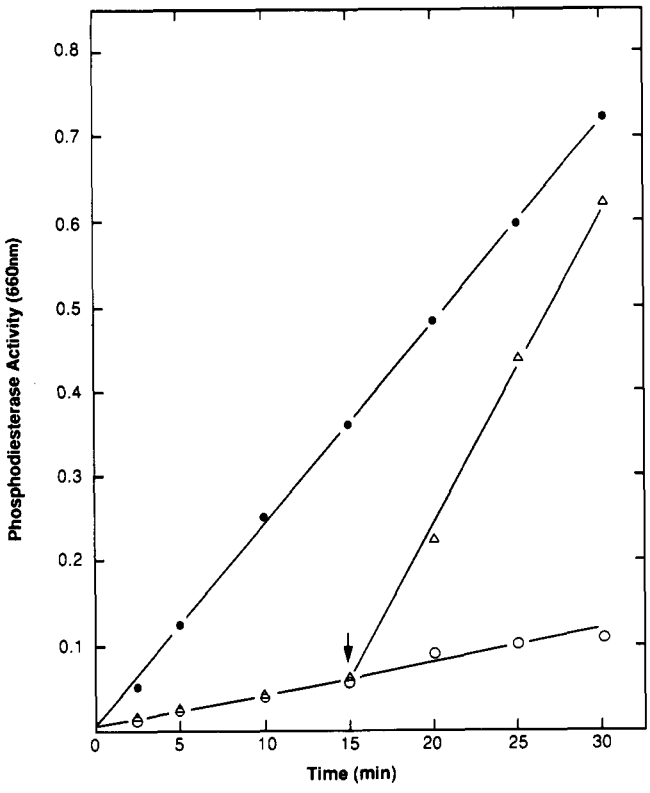

FIGURE 5: Reversal of the ginsenoside Rc inhibition of calmodulin-dependent phosphodiesterase by calmodulin. CaMPDE reactions with 0.04 unit/mL BHCaMPDE and 100 ng/mL calmodulin were performed in the absence (●) or presence (○, △) of 15 μg/mL ginsenoside Rc. Aliquots were removed at various time intervals as described under Experimental Procedures. After 15 min, calmodulin was added (△) to a final concentration of 10 μg/mL, and the progress of the reaction was followed.

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