Cyclic Nucleotide Phosphodiesterases as Possible Targets for Ginsenosides

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ABSTRACT

Cyclic nucleotide phosphodiesterases (PDEs) represent the unique enzymatic system degrading cAMP and cGMP which play a major role in the regulation of cell physiology. To investigate a possible molecular mechanism of ginsenosides, their activities were evaluated on PDEs which are recently described as new therapeutic targets. PDEs are classified into 7 families according to their genes (PDE1 to PDE7) and are differently distributed in tissues.

The IC_{50} values of ginsenosides were determined on PDE1 to PDE 5 chromatographically isolated from bovine aorta. The results show that total ginseng saponin extract preferentially inhibits PDE1 and PDE4 at concentrations nearby 200 μg/ml. Protopanaxadiol (PPD) fraction acts preferentially on PDE4 with and IC_{50} value of 100 μg/ml and inhibits also PDE1 and PDE5 at 14 to 2 fold higher concentrations, respectively. Protopanaxatriol (PPT) fraction preferentially inhibits PDE1 with and IC50 value of 170 μg/ml. Compound Rg1, originated from PPT fraction, and RG3 (S) represent the most active compounds towards PDE1 with IC_{50} values around 80 μM. However Rg3 (R), epimer of Rg3 (S) has no effect on the various PDEs tested, excepted on PDE3 wich is sligthly sensitive. Compound Rb1, originated from PPD, acts on both PDE1 and PDE4. It is two fold less active than Rg1 and Rg3 (S) on PDE1.

Taken together, these results mainly suggest that PDE1 and PDE4 inhibitions could be a molecular mechanism which would participate in ginsenoside mechanisms, especially the effect of PPD on blood vessel and on CNS.

Introduction

Cyclic nucleotide phosphodiesterases (PDEs) by hydrolyzing specifically cyclic AMP (cAMP) and cyclic GMP (cGMP) play a major role in cellular physiology. Their specific inhibitors are considered as new potential drugs especially for CNS and inflammatory diseases. This enzymatic system is constituted by 7 families according to their genes (PDE1 to PDE7; for review see Beavo, 1995 and Stoclet *et al.*, 1995). They are differently distributed in tissues and characterized by different substrate affinities and sensitivities to endogenous effectors (cGMP and calcium-calmodulin com-

plex).

Among these families, PDE4 is currently considered as intracellular target for new anti-inflammatory drugs (Teixeira *et al.*, 1997) and CNS drugs, PDE1 for new anti-proliferative drugs (Jiang et al., 1996; Vemulapalli *et al.*, 1996), PDE3 for cardiotonic drugs (Harrison *et al.* 1986) and PDE5 for impotence (Viagra®, Boolel *et al.*, 1996).

Ginseng, in which major active components are the ginsenosides, is used in traditional Chinese medicine to enhance stamina and capacity to cope with fatigue and physical stress (Gillis, 1997). Its mechanism of action remains unclear, although extensive literature deals with effects on CNS (memory, learning, behavior), cardiovascular system, immune function and metabolism. It was recently suggested that ginseng would be able to enhance nitric oxide synthesis in endothelial cells (Gillis, 1997). In the same way, we have previously shown that a specific inhibitor of PDE4 is able to stimulate NO production in vascular endothelial cells (Kessler & Lugnier, 1995) and that PDE3 and PDE4 synergistically participate to the regulation of the cardiovascular system (Stoclet *et al.*, 1995). Therefore, it could be hypothetized that ginseng effects could be related to PDEs wich are differently distributed in various cells and tissues.

To investigate the possible mechanism of action of ginseng and ginsenosides, their IC50 values were determined on PDE1 to PED5 isolated chromatographically from bovine aorta in comparison with reference drugs.

Materials and Methods

All chemicals were of analytical grade and were purchased from Sigma

Chemical company. [^{3}H]-cAMP or [^{3}H]-cGMP were obtained from Amersham. Rolipram and pentoxifylline, were a generous gift of Schering (Berlin), Hoescht (Paris) respectively. Cilostamide was synthetized as previously described (Lugnier *et al.*, 1985). Total ginseng saponin extract, protopanaxadiol, protopanaxatriol, Rg3 (R), Rg3 (S), Rg1, Rb1 were obtained from Korea Tobacco and Ginseng Research Institute by Dr. ND Kim.

Cytosolic PDE isoforms (PDE1, PDE3, PDE4 and PDE5) were purified by anion exchange chromatography from the media layer of bovine aorta by a modification (Komas *et al.*, 1991) of a method previously described (Lugnier *et al.*, 1986). Cytosolic PDE2 was isolated from cultured bovine aortic endothelial cells according to Lugnier and Schini, 1990.

PDE activities were measured by radioenzymatic assay as previously described (Keravis *et al.*, 1980) at a substrate concentration of 1μM cAMP or cGMP in the presence of 15000 cpm [^{3}H]-cAMP or [^{3}H]-cGMP as s tracer, respectively. The buffer solution was of the following composition: 50 μM TrisHCl pH 7.5, 2 μM magnesium acetate, 1 mg/ml bovine serum albumin and 1 μM ethylene glycol bis (β-aminoethylether) N,N,N^1,N^1 tetraacetic acid (EGTA). PDE1 was assayed in calmodulin-acti-

vated state (with 10μM CaCl2 and 18nM calmodulin insteas of 1mM EGTA) using [^{3}H]-cAMP as substrate. [PDE2 was assayed in the cGMP activated state (with 5 μM cGMP) using [^{3}H]-cAMP. To prevent the influence of cross-contamination between isolated PDE3 and PDE4, the studies, performed with [^{3}H]-cAMP as substrate] were always carried out in the presence of 10 μM rolipram or 100 μM cGMP, respectively.

The compounds were dissolved in DMSO; the final concentration of DMSO in the assay (1%) did not affect PDE activity. The studies were limited to compound concentrations of either to 300 μM (compound) or to 300 μ/ml (extract). The inhibition study on PDE activity included 6 concentrations of the drug. The results are expressed as percentage of inhibition of substrate hydrolysis. The IC_{50} values were calculated by non linear regression (Prism software) and represented the mean value of 3 determinations. The experimental error was about 15%.

Results and Discussion

We have first studied the effects of some reference PDE inhibitors on the various isolated PDEs (Table 1) to compare them with ginseng compounds (Table 2 and 3).

Table 1: Inhibitory effects of reference compounds on isolated PDE isozymes (IC_{50}, μM)

Compound substrate	PDE1 cGMP	PDE2 cAMP	PDE3 cAMP	PDE4 cAMP	PDE5 cGMP
Natural compounds					
caffeine	968	n.d.	890	1130	>>5000
Theophylline	220	226	310	520	538
papaverine	92	5.7	2.7	2.8	2.9
Synthetic coumpounds					
pentoxifylline	236	119	84	135	74
denbufylline	133	208	>>500	0.76	5.4
IBMX	4.3	8.1	10	24	12
nimodipine	3.2	n.s.	n.s.	24	n.s.
EHNA	n.s.	n.s.	3.4	n.s.	n.s.
cilostamide	n.s.	n.s.	0.15	133	165
rolipram	n.s.	n.s.	n.s.	1.1	n.s.
zaprinast	39	80	n.s.	123	1.7

The values represent the mean of three determinations with an experimental error of about 15%; n.s. indicates that the IC_{50} value is >200 μmM; n.d. indicates that IC_{50} value was not determined.

Caffeine, theophylline and papaverine, which are compounds originated from plants, inhibit differently the various PDE isoforms. Caffeine is the less effective, acting only in the millimolar range. Theophylline inhibits the various PDE isozymes at a lower range (200 to 500 μM). Papaverine

inhibits preferentially the calmodulin-independent isozymes (PDE2 to PDE5) in the micromolar range, but acts at higher concentrations on PDE1. Pentoxifylline, denbufylline and isobutyl-methyl xanthine (IBMX) which are xanthine analogues of theophylline, are more potent than theophylline. Pentoxifylline acts similarly on the different isoforms in the range of 100 to 200 μM. Denbufylline preferentially and potently inhibits PDE4 below the micromolar range and PDE5 in the micromolar range. IBMX, known as a nonselective PDE inhibitor, inhibits all tested PDEs in the tenth micromolar range.

Nimodipine preferentially acts on PDE1 in the micromolar range, and at higher concentrations on PDE4. Compound erythro-hydroxy-nonyl adenine (EHNA) specifically inhibits in the micromolar range the activation of PDE2 by cGMP.

Cilostamide specifically inhibits PDE4 whereas zaprinast inhibits PDE5 in the micromolar range

Table 2. Inhibitory effects of ginsenoside extracts on isolated PDE isozymes (IC_{50}, μg/ml)

Compound substrate	PDE1 cGMP	PDE2 cAMP	PDE3 cAMP	PDE4 cAMP	PDE5 cGMP
Total ginseng	195	20%*	28%*	220	45%*
protopanaxadiol	143	27%*	32%	100	200
protopanaxatriol	170	27%*	280	270	45%*

The IC_{50} values represent the mean of three determinations with an experimental error of about 15%. *Percentage of inhibition determined at 300μg/ml.

Table 2 shows that total ginseng saponins, protopanaxadiol and protopanaxatriol ginsenosides inhibit PDE1 similarly to theophylline with IC_{50} values all around 200 μg/ml. Total ginseng saponins preferentially and similarly inhibit both PDE1 and PDE4. Protopanaxadiol ginsenosides (PPD) act differently than total ginseng since they preferentially inhibit PDE4, although acting on PDE1 and PDE5 to a lesser extend. Protopanaxatriol ginsenosides (PPT) has a similar specificity for PDE1 as PPD but they act also on PDE3 and PDE4 around 300 μg/ml.

Table 3 reports the results obtained with isolated ginsenosides. Curiously Rb1, originated from PPD, acts similarly (IC_{50} values about 200 - 300 μM) on PDE1 and PDE4 suggesting that other components from PPD could be responsible for the PPD effects on PDE4 and PDE5. The Rb1 potency

Table 3. Inhibitory effects of purified ginsenosides on isolated PDE isozymes (IC_{50}, μM, or μg/ml)

Rb1	180(199)	18%	14%	268(297)	36%
Rg1	88(70)	42%	327(261)	258(206)	42%
Rg3(R)	0%	0%	28%	7%	2%
Rg3(S)	80(63)	20%	95(73)	84(64)	31%

The IC_{50} values represent the mean of three determinations with an experimental error of about 15%. *Percentage of inhibition determined at 300 μM.

on PDEs is similar to the theophylline one. Compound Rg1, originated form PPT, preferentially inhibits PDE1 (88μM) with the same potency as papaverine and inhibits other PDE isoforms at 300μ M.

Rg3 (R) and Rg3 (S) epimers have an inhibitory effect depending on their structure, Rg3 (S) being markedly more potent than Rg3 (R). This stereoselective effect is especially observed on PDE1 which is the most sensitive PDE isoform (IC_{50} value of 80 μM). These results fit well with the stereoselective effect of Rg3 observed at similar concentrations on tumor metastasis in mice (Mochizuki *et al.*, 1995). Rg3 (S) and Rg1 have similar pharmacological profiles on PDE isoform, Rg3 (S) being 3 fold more potent than Rg1 on PDE3 and PDE4.

The results clearly show that ginsenosides are able to inhibit various PDE isoforms depending on their origine and their structure. They mainly preferentially act on PDE1 and PDE4, especially total ginseng saponin extract and Rb1 at concentrations ranging 200μg/ml. PPD has a different profile since it preferentially inhibits PDE4 and acts also significantly on PDE5.

These effects on PDEs may privileged anti-inflammatory and anti-proliferative effects of ginsenosides as reported for PDE4 (Teixeira *et al.* 1997) and PDE1 inhibitors (Jiang et al., 1996; Vemulapalli *et al.*, 1996). They will also favour beneficial effect on CNS in which PDE1 and PDE4 are predominant. In the same way, it was shown that PDE4 inhibitor, an antidepressant compound, reduces neuronal damage following cerebral ischemia (Kato *et al.*, 1995). The effect of Rg1 that is one of the most potent we observed on PDE1 is in agreement with the results obtained by Stancheva et Alova (1993) in rat brain and suggests that it is merely PDE1 which is implicated in the later study.

Rg3 (S) is the most potent assayed ginsenoside on PDE4. Its potency is similar to that of pentoxifylline which is a non-selective PDE inhibitor (see Table 1) known as an anti-inflamatory drug, since it decreases TNF-α and IL-1β production by inflammatory cells (Strieter *et al.*, 1988). Therefore, it could be suggested that Rg3 (S) might act as an anti-inflammatory drug.

Our previous studies performed on isolated rat aorta with PDE3 and PDE4 inhibitors, showed that PDE3 inhibitors are endothelium-independent relaxing agents, whereas PDE4 inhibitors are endothelium-dependent relaxing agents (Komas *et al.*, 1991). Moreover, we have demonstrated that NO produced by endothelial cells induces a synergistic effect between PDE3 and PDE4 inhibition mediated by cGMP (Lugnier and Komas, 1993; Eckly and Lugnier, 1994). Therefore it could be suggested that the endothelium-dependent-relaxing effect observed for some ginsenoside compounds which act on PDE4 could be related to PDE4 inhibition. Similarly, we have shown that PDE3 potentiates PDE4 inhibition, inducing a positive inotropic effect (Muller *et al.*, 1990). Ginsenoside compounds such as PPT, Rg1 and Rg3 (S) by inhibiting both PDE3 and PDE4, have some potentialities to act positively on cardiac contraction and on vasodilatation.

Moreover, it was recently shown that PDE4 inhibition induces apoptosis of carcinoma cells (Marko *et al.,* 1998). This effect is in accordance with the cancer chemopreventive effect of red ginseng (Xiaoguang *et al.,* 1998) and suggests that effects of ginsenosides could be related to PDE4 inhibition.

Extract of Korean red ginseng is able to relax corpus cavernosal smooth muscle (Choi *et al.,* 1998). Furthemore, PPD is able to inhibit PDE5 which is a new target for impotence (Boolell *et al.,* 1996) and PDE4 which is target for endothelium-dependent vasodilation. These data strenghten the hypothesis that ginsenosides act via PDE inhibition.

In conclusion, the interaction of the different ginsenosides with various PDEs known to regulate many physiological processes suggests that PDE inhibition may participate in ginsenosides effects and that PDE isoforms could be potential targets for ginsenosides.

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