THE RECIPROCAL EFFECTS OF SEVERAL GINSENOSIDES ON THE ADENYLATE CYCLASE AND GUANYLATE CYCLASE

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INTRODUCTION

Many works have been carried out on the chemistry, and physiological and pharmacological action of the ginseng components (Han, 1978; Kim, 1978). Recently, extensive interest is increasing in the studies of specific effects of the ginseng components in vitro (Joo et al., 1973; Kim, 1978; Kim et al., 1970, Oura et al., 1974, Joo, 1984; Oura, 1983; Yokozawa, 1979). We have been working for several years on the effects of some ginsenosides on the activities of adenylate cyclase and guanylate cyclase. We have observed that several ginsenosides activiate or inactivate the two enzymes reciprocally. (Seo et al., 1983). In the present work, we report the finding that GMP reverses the ginsenoside Rb2-induced inhibition of the adenylate cyclase, and also the finding that ginsenoside acts like a hormone-like substance.

MATERIALS AND METHODS

1. Materials

Dried ginseng root of six years old was purchased from the market. Cyclic AMP assay kit and cyclic GMP assay kit were the products of Radiochemical Center (Amersham, England).

2. Extraction and Purification of Ginsenosides.

Extraction of ginsenosides was performed according to the Oura's method (Oura and Hiai, 1974). Before following the Oura's procedure, the filtrate extracted with Tris-HC1 buffer (pH 7.6) was freeze-dried and the resulting powder was dissolved in methanol and then the solution was treated with ether.

The final freeze-dried extract was applied to TLC on silica gel plate (Seo et al., 1983a).

3. Preparation of Particulate Adenylate Cyclase and Particulate Quanylate Cyclase.

Rats, weighing 150-200g, were supplied by the Animal Room of Seoul National University. Adenylate cyclase and quanylate cyclase from the rat brain were prepared according to the methods of Sutherland (Johnson et al., 1974), and of Nakazawa and Seno (1974). The protein content of the enzyme preparation was determined by the method of Lowry et al. (1951). Adenylate cyclase and guanylate cyclase preparation contained about 2.57mg and 2.40mg protein per milliliter, respectively.

4. ^{125}I-Iodination of Ginsenoside Rb_2 and the Purification of the Labeled ^{125}I-Rb_2.

As reported previously (Seo et al., 1983a),

^{125}I-iodination was carried out by using iodogen (1,2,4,6-tetrachloro-3a, 6a-diphenyl glycouril) according to the method of Fraker and Speck (1978). ^{125}I-Rb2 was separated from the reaction mixture as described in Seo et al. (1983a).

5. Assay of Binding of ^{125}I-Rb_2 on the Cells.

^{125}I-Rb_2 binding on the fibroblast SCK tumor cells was measured in the presence and in the absence of dopamine, one of the neurotransmitters specific to D-1 dopamine receptor. SCK tumor cells were kindly donated by Dr. Man-sik Kang, Department of Zoology, Seoul National University. The assay procedures are described in Seo et al. (1983a).

6. Radioimmunoassay of cAMP and cGMP

cAMP and cGMP formed under the various reaction conditions were radioimmunoassayed as reported previously (Seo et al., 1983b), using cAMP and cGMP assay kits.

RESULTS AND DISCUSSION

1. Reciprocal Effects of Ginsenosides on the Activities of Particulate Adenylate Cyclase and Particulate Guanylate Cyclase from the Rat Brain.

Fig. 1 shows that each of ginsenosides Rb_2, Rb_1, and Rc exerts reciprocal effects on the activities of particulate adenylate cyclase and particulate guanylate cyclase in the range of concentration through 0.3mM to 3.0mM of ginsenosides. The reciprocity of the effect of each ginsenoside on the two enzymes varies from one ginsenoside to another. For example, the conditions that inhibit the adenylate cyclase in maximum are 0.8mM for ginsenoside Rb_2 (49% inhibition), 0.3mM for ginsenoside Rb_1 (47% inhibition), and 0.1mM for ginsenoside Rc (83% inhibition), similar variation of the conditions for the maximum activation of guanylate cyclase can be seen in Fig. 1.

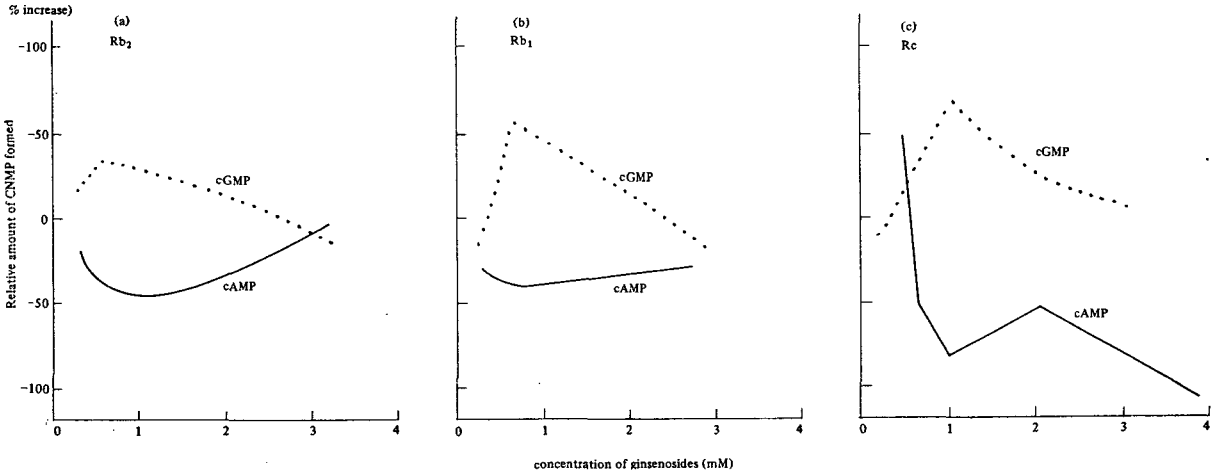


Fig. 1. Effects of ginsenosides on the activities of particulate adenylate cyclase and particulate guanylate cyclase from the rat brain.
(a), Rb_2; (b), Rb_1; (c), Rc.

Reciprocal action of ginsenoside Rb_2, Rb_1, and Rc suggests that these substances may be interesting regulatory effectors or hormone-like substances. Furthermore, the variation of reciprocity patterns for individual ginsenosides means that the effects of the ginsenosides are ginsenoside-specific.

Ginsenoside Re also exerts a reciprocal action to adenylate cyclase and guanylate cyclase in the range of very low concentration through about 0.02mM to 0.1mM (data are not shown). In contrast to the reciprocal actions of ginsenosides Rb_2, Rb_1, and Rc, it was found that ginsenoside Rd, Rf, and Rg_1 do not exert the reci-

procal effects on adenylate cyclase and guanylate cyclase (data are not shown).

2. Competitive Binding of Ginsenoside Rb_2 and Dopamine on the Cells.

In order to understand the binding nature of ginsenoside Rb_2 on the cells, and also to see if ginsenoside Rb_2 is really a hormone-like substance, competition between ginsenoside Rb_2 and dopamine in binding onto the cells, was studied using ^{125}I-Rb_2. The cells used for this experiment were from fibroblast SCK tumor cells of mouse which are originated spontaneously and characterized by Kang et al. (1980). As is shown in Fig. 2, ginsenoside Rb_2 binds in substantial amount in the absence of dopamine showing a maximum value at 15 minutes incubation, whereas binding was reduced strikingly in the presence of dopamine. From this observation, it seems to be very likely that ginsenoside Rb_2 might bind to D-1 dopamine receptors of the cells (Kebabian and Calne, 1979). Recently, the crude extract of saponin was found to bind to the receptors *in vitro* [see Oura's Review (Oura, 1983)].

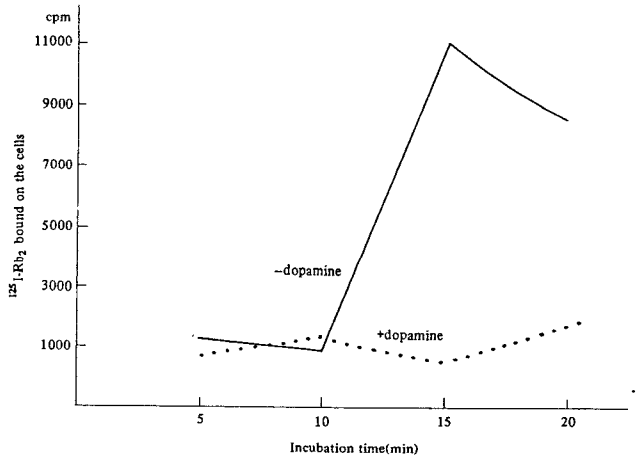
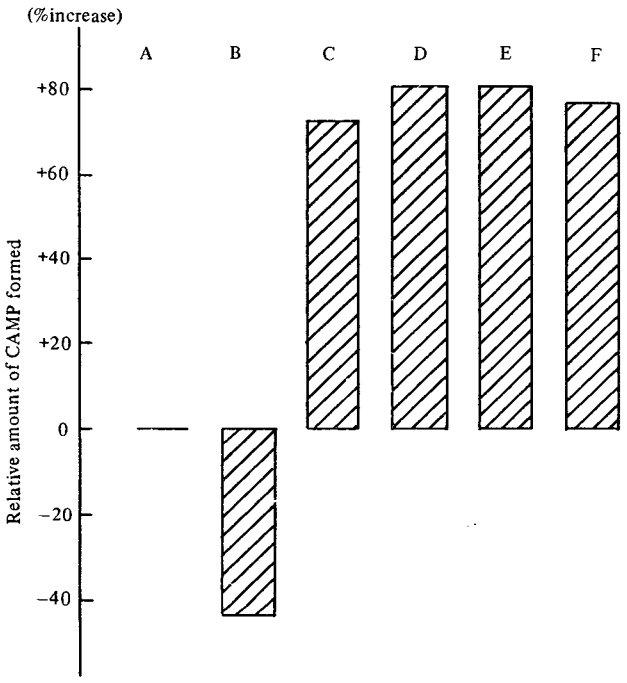


Fig. 2. Competition between ginsenoside Rb_2 and dopamine in binding onto the cells. Binding of ^{125}I-Rb_2 in the absence (———) and in the presence (. . . . .) of dopamine. The cells used for this experiment were from SCK fibroblast tumor cell which is originated spontaneously and characterized by Kang et al. (1980).

3. GMP as a Positive Effector of Adenylate Cyclase.

As shown in Fig. 3, inhibitory effect of ginsenoside Rb_2 on the activity of adenylate cyclase can be reversed by the incubation of adenylate cyclase with GMP. This figure also shows that GMP activates the ginsenoside Rb_2-inhibited adenylate cyclase at the different concentrations of ginsenoside Rb_2 almost to the same degree. This finding suggests that GMP, like fluoride, acts as a positive effector to adenylate cyclase inhibited by ginsenoside Rb_2. A possible explanation for the activation of Rb_2-inhibited enzyme is that Rb_2-induced conformational change of the enzyme may be restored, or, the native comformation of the enzyme may be transformed to the more active form. It is interesting to note the findings of Downs et al. (1980) that treatment of



A; minus GMP; minus Rb_2
B: minus GMP; Rb_2 (10^{-4}M)
C: GMP (0.01mM); minus Rb_2
D: GMP (0.01mM); Rb_2 (10^{-6}M)
E: GMP (0.01mM); Rb_2 (10^{-5}M)
F: GMP (0.01mM); Rb_2 (10^{-4}M)

Fig. 3. Reversion of ginsenoside Rb_2-induced inhibition of the activity of adenylate cyclase by the addition of GMP.

the erythrocyte membrane with isoproterenol and a high concentration of GMP (about 1mM) makes the adenylate cyclase more sensitive to activation by Guopp[NH]P and insensitive to activation by fluoride. However, it should be noted that, unlike ginsenoside Rb_2, isproterenol is β-adrenergic.

Fig. 4 shown the activation of ginsenoside Rb_2-inhibited adenylate cyclase by the increasing concentration of GMP. The results indicate that increase of activation of Rb_2-inhibited enzyme is not proportional to the amount of GMP added.

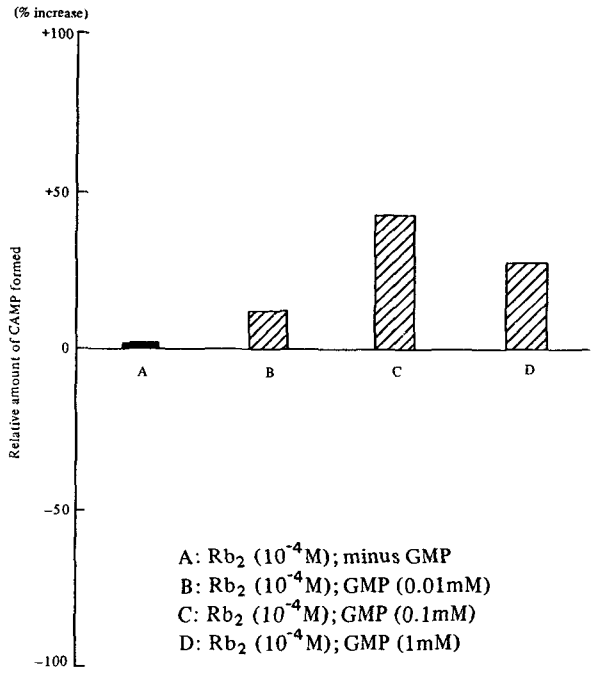


Fig. 4. Activation of ginsenoside Rb_2-inhibited adenylate cyclase by the addition of increasing amount of GMP.

In order to obtain some information for the action mechanism of GMP on the adenylate cyclase, activities of the enzyme not treated with ginsenoside Rb_2 was measured under the different conditions as indicated in Fig. 5. It can be seen that GMP activates the enzyme, even when the enzyme is not ginsenoside Rb_2-inhibited beforehand. This finding indicates that there are specific binding sites for GMP on the native enzyme molecule, and that the enzyme can be regulated alosterically by GMP. It has been known that regulatory nucleotides of the adenylate cyclase systems are GDP and GMP, but not GMP (Bradham and Cheung, 1982). Considering this well established fact, our findings appear rather interesting. Fig. 5 also shows that GMP and fluoride act synergistically when both of them are added together to the enzyme. This result suggests that GMP and fluoride have the two separate binding sites. This finding is contradictroy to that of Downs et al (1980). These reporters have suggested that binding of GMP on the enzyme makes it insensitive to fluoride. The different regulatory effects of GMP on the cells treated with isoproterenol or treated with ginsenoside Rb_2 may be due to the different binding specifities of the two substances as the ligands to the receptors; the former is β-adrenergic, whereas the latter is D-1 dopaminergic.

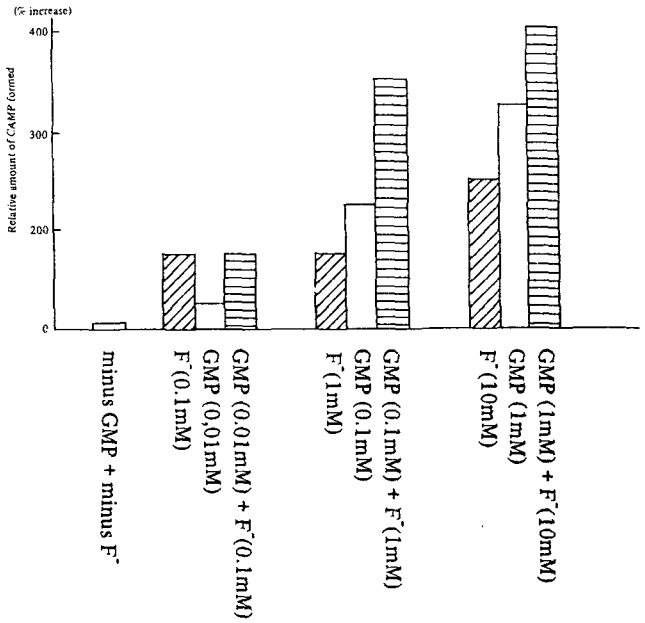


Fig. 5. Effects of fluoride and GMP on the activity of adenylate cyclase in the absence of ginsenoside Rb_2.

From the data presented here, it is concluded as follows. First, ginsenoside Rb_2, Rb_1, and Rc exert reciprocal effects on the activities of adenylate cyclase and guanylate cyclase from rat brain. The competition between ginsenoside Rb_2 and dopamine in binding onto the cells suggests that ginsenoside Rb_2 is a hormone-like substance specific to D-1 dopamine receptors. Second, GMP reverses the ginsenoside Rb_2-in-

duced inhibition of the adenylate cyclase. Synergism, effected by GMP together with fluoride, suggests that GMP binds to a site different from that of fluoride.

Chong: What is the clinical relevance of cyclic AMP and GMP on the brain of human beings?

Park: We did this experiment not *in-vivo*. It was carried out *in-vitro*.

몇가지 진세노사이드들이 Adenyl 산 고리화효소 및 Guanyl산 고리화 효소에 미치는 상반적인 효과

박인원(서울대 자연대 화학과)

쥐의 뇌에서 추출한 입자상 아데닐산 고리화효소와 입자상 구아닐산효소의 활동성에 미치는 몇 가지 진세노시드들의 효과를 조사하였다. Rb_2, Rb_1, Rc 및 Re들과 같은 약간의 진세노사이드들이 두 효소들의 활동성을 상반적으로 변화시키는 것을 관찰하였다.

아데닐산 고리화효소와 구아닐산 고리화효소의 활동성에 미치는 GMP 및 AMP의 조절작용을 조사하였다. 진세노시드 Rd로 방해된 아데닐산 고리화효소는 GMP를 첨가함에 따라서 활성화되었다. 마찬가지로, 진세노시드 Rb_2로 방해된 아데닐산 고리화효소도 GMP에 의해서 활성화되었다. 다른 한편, 진세노시드 Rc로 활성화된 구아닐산 고리화효소는 AMP 또는 GMP를 첨가함에 따라서 방해되었다.

진세노사이드 Rb_2와 도파민 사이에는 아데닐산 고리화효소 계상의 수용체들에의 결합에 있어서 경쟁적이라는 것을 알았다.

이 결과는 진세노시드 Rb_2가 세포의 D-1 도파민 수용체에 특이하게 결합한다는 것을 말해 준다.

REFERENCE

1. Bradham, L.S., and Cheung, W.Y. (1982). Prog. Nucleic Acid Res. Mol. Biol. *27*, 189.
2. Downs, R.W., Spiegel, A.M., Singer, M., Reen, S., and Aurbach, G.D. (1980). J. Biol. Chem. *255*, 949.
3. Johnson, R.A., and Sutherland, E.W. (1974). Methods in Enzymology *38*, 135.
4. Joo, C.N. (1984), Biochemical News *4*, 5.
5. Joo, C.N., Choi, R.S., Lee, S.J., Cho, S.H., and Son, M.H. (1973). Korean Biochem. J. *6*, 185.
6. Kang, M.S., Song, C.W., and Levitt, S.H. (1980). Cancer Res. *40*, 1130.
7. Kebabian, J.W. and Calne, D.B. (1979). Nature *277*, 93.
8. Kim, T.B., Han, S.H., Lee, S.H., and Kim, J.K. (1970). Korean Biochem. J. *3*, 35.
9. Kim, T.B., Kim, J.K., and Lee, K.B. (1970). Korean Biochem. J. *3*, 41.
10. Kim, N.D. (1978), Korean Ginseng, Korean Ginseng Research Inst., p. 115.
11. Lowry, O.H., Rosebourg, N.H., Fair, A.L., and Randall, R.J. (1951). J. Biol. Chem. *193*, 265.
12. Nakazawa, K., and Seno, M. (1974). J. Bio. Chem. *249*, 4207.
13. Oura, H. (1983). The Ginseng Review, *1*, 1.
14. Oura, H. and Hiai, S. (1974). Proc. International Ginseng Symp. p. 23.
15. Seo, K.L., Moon, J.G., Cha, M.K., Lee, K.S., Lee, S.Y., Lee, Y.Y., and I. Park (1983a), Korean J. Ginseng Sci. 7. 148.
16. Seo, K.L., Koh, M.J., Lee, Y.Y., I. Park, and Lee, S.Y. (1983b). Korean J. Ginseng Sci *7*, 95.
17. Yokozawa, T., Kitahara, N., Okuda, S., and Oura, H. (1979). Chem. Pharm, Bull. *27*, 419.

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