Korean J. Ginseng Sci.
Vol. 20, No. 2, 168~172(1996)

The Action of Triterpenoidal Glycosides of Dammarane Series and Their Aglycones on K^+ and H^+ Fluxes in Erythrocytes, Induced by Ionophore A_{23187} and Divalent Ions

Yu. A. Kim, Kyeong-Mee Park[1], Jong-Su Kyung[1], Hak-Chul Hyun[1], Yong-Bum Song[1], Han-Jae Shin[1] and Hwa-Jin Park[2]

Institute of Cell Biophysics, Russian Academy of Sciences, 142292, Pushchino, Moscow region, Russia.
[1]Korea Ginseng & Tobacco Research Institute, Department of Biochemical Pharmacology, Taejon 305-345, Korea
[2]Department of Medical Laboratory Science, College of Health Science, Inje University, Kimhae Kyongsangnam-Do 621-749, Korea
(Received March 3, 1996)

Abstract : Ginsenoside Rb_1, at a concentration of 10 μg/ml and over, initiated the cycle of oscillation of ion flux in erythrocytes after the cells had been treated with a protonophore, carbonyl cyanide p-trifluoro- methoxyphenyl hydrazone (FCCP) and then with a Ca^{2+} ionophore, A_{23187}. Its action was similar to the additional portion of Ca^{2+}-ionophore or Ca^{2+} ion to the erythrocytes.

Effects of Rg_1 and Rf were different from that of Rb_1. They did not induce the oscillation. They, however, increased the extracellular K^+ concentration and pH without returning to the initial state in the erythrocytes processed with FCCP and A_{23187}.

We established that ginsenosides from 20-(S)-panaxatriol family induced the membrane hyperpolarization in erythrocytes, which was attenuated by the pretreatment of Rb_1, a major component of 20-(S)-panaxadiol.

Key words : ginsenosides, Ca^{2+}-activated K^+ channel, erythrocytes, FCCP, A23187

Introduction

It is known that ginsenosides act on cellular membranes causing changes in the structure and permeability of membranes.[1] These substances can either produce hemolysis or protect from hemolysis.[2] Their activity for or aginst hemolysis depends on the molecular strucrure of these substances. The mechanism of hemolysis, with respect to the association with ginsenosides, is not yet clear. The change in ion permeability of erythrocytes is proposed to be associated. There is few information, in literature, about the influence of ginsenosides from ginseng on the regulation of ion fluxes across the membrane of erythrocytes and other cells.

In the suspension of intact erythrocytes processed by FCCP and then by A23187, an oscillation of ion flux across the membrane is produced. This process is the result of the periodic opening and closing of Ca^{2+}-activated K^+ channels.[3] Since the mechanism of oscillation has been investigated in details by many investigagtors, this phenomenon is a convenient model for the study of influences of various physico-chemical factors on erythrocytes membranes.

In this paper, we presented the result of actions of various components of total saponin from *Panax ginseng* C.A. Meyer; triter- penoidal glycosides of dammarane series, namely gin-

senosides Rb_1, Rb_2, Rg_1 and Rf, and their aglycons, namely 20-(S)-protopanaxatriol (PT) and 20-(S)-protopanaxadiol (PD), on the oscillation of ion fluxes in erythrocytes.

Materials and Methods

Ginsenosides were obtained from the analytic center of Korea Ginseng & Tobacco Research Institute (Taejon, Korea). Ionophores, carbonyl cyanide p-trifluoro-methoxyphenyl hydrazone (FCCP) and A_{23187} were purchased from Sigma (St. Louis, USA). The other chemicals used in the experiment were all reagent grade.

Freshly drawn blood from rats of Wistar's line with weights of 120~140 g was heparinized and centrifuged. Plasma and buffy coat were aspirated. The cells were washed twice with 150 mM NaCl solution and were resuspended in this same solution at 3~5℃. Hematocrit was 10%. H^+-sensitive glass electrode and K^+-selective membrane electrode (Orion, USA) were used.

Three thousands μl of unbuffered solution (150 mM choline chloride, 1 mM KCl, 0.15 mM $MgCl_2$, 5 mM glucose) was pipetted into a measuring chamber and maintained at 37℃. Erythrocytes suspension (200 μl) was transferred to the measuring chamber for stirring. After 30 μM FCCP was added to the chamber, the FCCP-mediated H^+ equilibrium across the erythrocytes membranes was achieved within few minutes. Ca^{2+}-ionophore, A_{23187} was then added for the initiation of ion flux oscillations in erythrocytes.

The presence of Ca^{2+}-activated K^+-selective ion channels has been demonstrated in the membranes of many types of cells.[4,5] Ginsenosides were added in the suspension of erythrocytes when the periodic opening and closing of K^+ channels were induced.

Results and Discussion

The membrane of vertebrate erythrocyte contains Ca^{2+}-activated K^+ channels.[6,7] Under physiological conditions, these channels are almost closed. When the intracellular concentration of Ca^{2+} exceeds the physiological level, the channels open and K^+ conductance of the erythrocyte membranes increases considerably.

Under the standard procedure stated in "Materials and Methods", the oscillation usually started within 5~15s after the addition of ionophore. Within the time resolution of the recording system, the initial increases in extracellular K^+ concentration and pH (membrane hyperpolarization) took places simultaneously.

The experiments were carried out under the condition when the oscillation of ion flux was ceased after the first cycle. K^+ channels were closed and membrane was hyperpolarized in that condition (Fig. 1).[8] Such states of cells are unstable and very sensitive to weak physico-chemical effects.

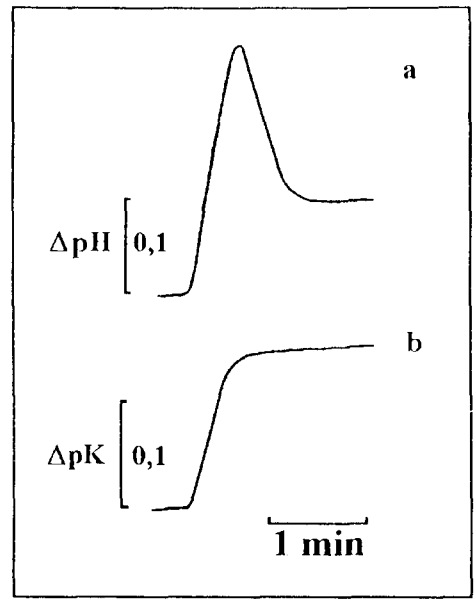

Fig. 1. Change in the concentrations of H^+ (a) and K^+ (b) ions in the suspension of erythrocytes induced by FCCP (30 μM) and then by A_{23187} (0.8 μM).

The addition of insignificant portion of Ca^{2+} ions resulted in opening of K^+ channels and produced the next cycle of oscillation (Fig. 2a). Glycosides and aglycones were added in the suspension of erythrocytes before or after the addition of ionophore A_{23187}.

1. Action of PT and PD

Fig. 2b illustrates the experiment in which PT, in a concentration of 30 μg/m*l*, initiates an oscillation of ion flux across the membrane of erythrocytes. As distinct from control, the system did not come back to the initial state after one cycle of oscillation. A slow hyperpolarization of the membrane was observed. PD, at this concentration, did not induce the generation of oscillation of ion flux in erythrocytes. A slow hyperpolarization of membranes was only observed (Fig. 2c).

It is possible that the effects of PT and PD reflect the distinctions in their molecular structures.

2. Action of Ginsenosides Rb_1, Rb_2, Rg_1 and Rf

Ginsenoside Rb_1, at concentration of 10 μg/m*l* and over, initiated the additional cycle of oscillations of ion flux across the membrane. Its action was similar to the introduction of additional portion of A_{23187} or Ca^{2+} (Fig. 3a). The addition of Rb_1 without ionophore A_{23187}, however, did not result in the induction of oscillation. The amplitude of hyperpolarization of the membrane depended on the concentration of ginsenoside (Fig. 4).

Ginsenoside Rb_2, at concentrations of 10~30 μg/m*l*, did not affect the initiation of oscillation of ion flux appreciably in erythrocytes. A slight increase of hyperpolarization of the membranes was only observed (data not shown).

Effect of ginsenoside Rg_1 was different from that of Rb_1. Rg_1, at a concentration of 5 μg/m*l* and over, did not initiate the oscillation of ion flux. It increases, however, the extracellular K^+

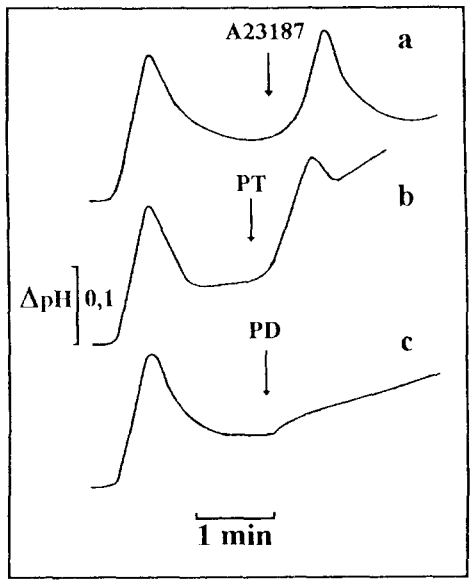

Fig. 2. Change in pH of the suspension of erythrocytes in the presence of: a) A_{23187} in the concentration of 0.5 μM; b) 20-(S)-protopanaxatriol in the concentration of 30 μg/m*l*; c) 20-(S)-protopanaxadiol in the concentration of 30 μg/m*l*, after one cycle of oscillations of H^+ and K^+ ions.

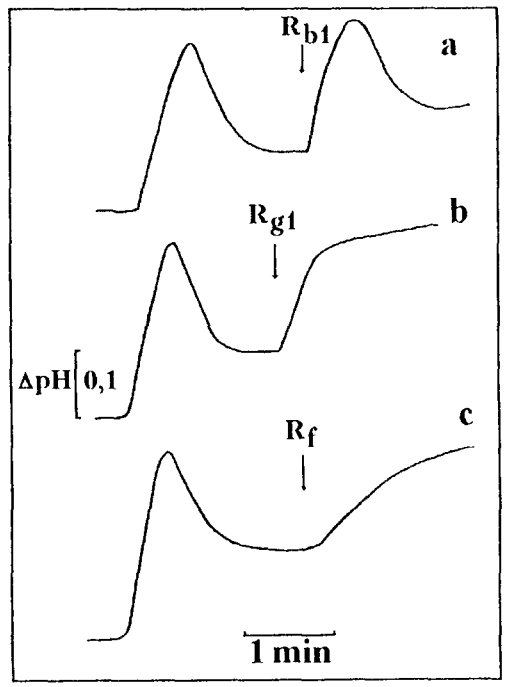

Fig. 3. Induction of oscillation of H+ flux across the membrane of erythrocytes in the presence of (a) 20 μg/m*l* of ginsenoside Rb_1, (b) 5 μg/m*l* of Rg_1, or c) 50 μg/m*l* of Rf.

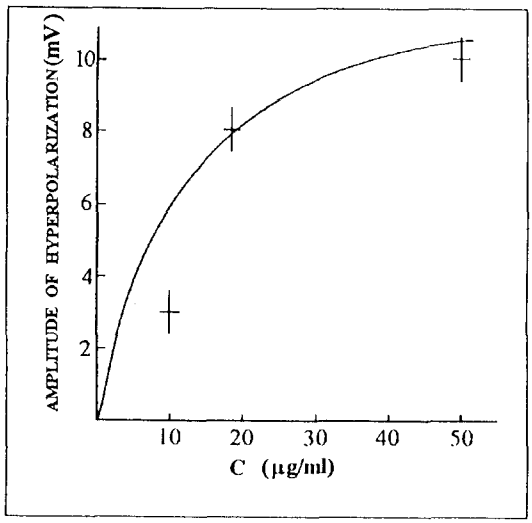

Fig. 4. The dependence of hyperpolarization on the concentrations of Rb_1.

concentration and pH without returning the system to the initial state (Fig. 3b).

As in the case with ginsenoside Rg_1, ginsenoside Rf did not generate the oscillation of ion flux at concentrations of 10~100 μg/m*l*. Rf produced hyperpolarization of the membrane depending on its concentration (Fig. 3c).

It is known that concentrations of ionophore A_{23187} and Ca^{2+} have substantial significances in the initiation of oscillation of ion flux. Under our experimental condition, only one cycle of oscillation was initiated and system returned to its initial level. The cells under such a critical condition can be passed into other states at slight changes of its parameters. The Ca^{2+} ion flux into the cells, at that situation, is critically important in the generation of oscillation.

It is necessary to increase intracellular Ca^{2+} concentration for the generation of subsequent cycles of oscillation. It can be done by the introduction of an additional quantity of ionophore A_{23187} or Ca^{2+} into the extracellular medium. Ca^{2+} influx becomes larger in comparison with Ca^{2+} efflux produced by Ca^{2+}-ATPase.

For explaining the action of glycosides, we can propose several mechanisms. First, ginsenosides Rg_1 and Rf may form, in erythrocytes membranes, the uncontrolled ion-conducting structures through which an efflux of cellular K^+ takes place, resulting in the hyperpolarizaton of membranes. The registered increase of extracellular pH is connected with the transfer of H^+ by FCCP in the direction of electrical field. In this case, the oscillation is not registered since the flux of K^+ ions is not induced by Ca^{2+}-activated K^+ channels.

On the other hand, it is possible that ginsenosides Rg_1 and Rf inhibit ATP-dependent Ca^{2+}-pump transferring Ca^{2+} from cells into external medium. The Ca^{2+} influx, then, exceeds the Ca^{2+} efflux resulting in the increase of intracellular Ca^{2+} concentration and the activation of Ca^{2+}-sensitive K^+ channels. This assumption is, however, in contrast with the data of Li-Qing and Lin,[9] who showed that Rg_1 did not inhibit the Ca^{2+}/Mg^{2+}-ATPases of synaptasoms up to the concentration of 200 μg/m*l*.

It is possible to assume that ginsenosides Rg_1 and Rf affect the system of anion exchanger changing the distribution of Cl ions in medium. It is known that the pulsing increase of Cl concentration results in the activation of K^+ efflux and hyperpolarization of the membrane.[9]

It should not be excluded that the direct action of ginsenosides Rg_1 and Rf on the controlling system of K^+ channels results in the increase of the probability of the channels being in the open states. In this case, the efflux of K+ ions from cells increases inducing further events described earlier.

Ginsenosides of PD differ from ginsenosides of PT by their action on the membranes. Rb_1 generated the oscillation of ion flux with return of the system to the initial state. PT also generated one cycle of oscillation but failed in returning the system to the initial state.

According to the mechanism of generation of oscillation, the addition of PD ginsenoside results

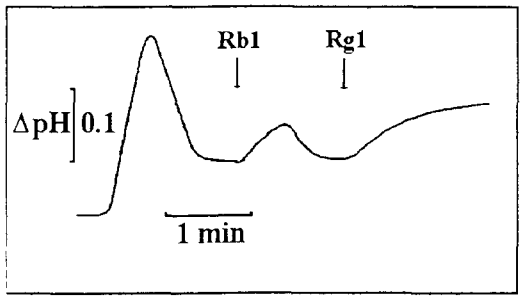

Fig. 5. The influence of Rg_1 (5 μg/m*l*) on the membrane of erythrocytes after preliminary treatment of Rb_1 (10 μg/m*l*).

in an increase of intracellular Ca^{2+} concentration up to levels sufficient for the opening of Ca^{2+}-activated K^+ channels. The characteristic processes occurring in the generation of ion oscillation are further developed.

It was reported that ginsenosides of PT have hemolytic activity while some ginsenosides of PD have protective activity against hemolysis.[2] The mechanism of these phenomena is not completely clear. It is considered that one of the ways resulting in hemolysis of erythrocytes is the infringement of ion permeability of cell membranes (It is possible that the infringement of ion regulation system of the cell, particularly, Ca^{2+}-activated ion channels precedes the hemolysis of cells.).

We have established that the effect of ginsenosides, being hemolytic, on the amplitude of hyperpolarization is attenuated considerably after the preliminary treatment of Rb_1 (Fig. 5). If the infringement of ion regulation of cell membranes precedes the hemolysis of cells in the presence of PD ginsenosides, the action of Rb_1 may be explained in the context stated just above for its antihemolytic effect.

For the studies of the action mechanisms of ginsenosides on ion fluxes across the membrane of erythrocytes in detail, the complementary research with the use of inhibitory analyses is nessesary.

요 약

적혈구 현탁액에 수소운반물질인 FCCP와 칼슘운반물질인 A_{23187}를 순차적으로 첨가하면 막을 사이에 한 이온들의 재평형상태가 이루어진다. 이 상태에서 진세노사이드 Rb_1을 10 μg/m*l* 이상의 농도로 처리했을 때, 이온흐름의 교란이 관측되었다. 진세노사이드 Rb_1의 이러한 효과는 칼슘운반물질이나 Ca^{2+}이온을 적혈구세포에 재첨가한 것과 비슷한 양상을 보였다. Rg_1과 Rf의 경우에는 Rb_1의 경우와 상당히 차이를 보였는데, 이 두 진세노사이드는 이온교란을 일으키지 않았다. Rg_1과 Rf의 첨가에 의해 적혈구 세포외액 중의 K^+이온의 농도와 pH의 증가가 일어 났으며, 막이 완만한 과분극상태에 도달했다. 용혈을 일으키는 것으로 보고되어 있는 PT계의 진세노사이드는 막을 과분극상태로 만드는데, Rb_1의 전처리에 의해 이러한 막의 과분극 정도가 억제되었다.

References

1. Nakamura, T., Inoue, K. and Nogima, S. : *J. Pharm. Dyn.* **2**, 374 (1979).
2. Namba, T., Yoshizaki, M., Tomimori, T., Kobashi, K., Mitsui, K. and Hase, J. : *Chem. Pharm. Bull.* **21**, 459 (1973).
3. Vestergaard-Bogind, B. and Bennekou, P. : *Biochim. Biophys. Acta*, **688**, 37 (1982).
4. Hoffman, E. K. and Simonsen, L. O. : *Physiol. Rev.* **69**, 315 (1989).
5. Hunter, M., Lopes, A. G., Bouplaep, E. and Giebish, G. : *Am. Physiol.* **251**, 725 (1986).
6. Gardos, G. : *Acta Physiol. Sci. Acad. Hung.* **10**, 185 (1956).
7. Lew, V. L. and Beauge, L. : *Transport across Biological Membranes*, Vol. 2, Springer-Verlag, Berlin and New York, p. 81 (1979).
8. Sadykov, Y. H. : *Post. doc. degree dic.* Institute of Biophysics, Pushchino, USSR, p. 123 (1985).
9. Li-Qing, J. and Lin, S. : *Acta Pharmacologica Sinica*, **12**, 504 (1991).