EFFECT OF GINSENG SAPONIN ON THE VASCULAR SMOOTH MUSCLE

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Abstract

Aortic strips were prepared from rabbits, and the tensions were maintained by administration of norepinephrine into the incubation chamber. The application of diol or triol induced relaxation of the aortic strip, as indicated by the decreased aortic tension. Triol, in a concentration of 30 mg % causes approximately 50% of muscle relaxation, whereas a similar degree of relaxation is induced by 50 mg % of diol. This indicates that both triol and diol cause relaxation of the aorta, but that triol is about 170% more potent than diol.

It is well established that blood-vessel smooth-muscle tone is regulated by the available intracellular Ca^{++} concentration, which in turn is profoundly influenced by interaction of the cellular membrane and sarcoplasmic reticulum in the smooth muscle. Thus, any agent which modifies the smooth-muscle tone is expected to interfere with the Ca^{++} binding or uptake of sarcolemma and sarcoplasmic reticulum.

In the following experiments sarcoplasmic reticulum and sarcolemma were prepared from the ventricle of rabbit heart, and the active Ca^{++} uptake by these cellular components was measured employing Ca^{45} in the presence of triol and diol.

It was found that the active Ca^{++} uptake in the presence of ATP by sarcoplasmic reticulum was inhibited by both triol and diol. Panaxatriol, in a concentration of 80 mg %, inhibited Ca^{++} uptake by 30%, whereas panaxatriol in the same concentration inhibited uptake by 20%. It is clear that triol is a more potent inhibitor of active Ca^{++} transport in sarcoplasmic reticulum than diol.

The Ca^{++} binding of the cellular membrane was also studied employing Ca^{45} and milipore techniques. It was found that triol in a concentration of 80 mg % decreased Ca^{++} binding by 29%. Diol in the same concentration decreased the binding by 17%. It is clear that both triol and diol inhibit Ca^{++} binding to the cellular membrane, but triol is approximately 180% more potent than diol.

Introduction

The blood pressure is regulated by the smooth muscle tone of blood vessels. The degree of constriction of blood vessel wall is expected to determine the blood pressure. Thus the effect of drugs on the blood pressure could be analysed by investigating the effect of these drugs on the smooth muscle of blood vessels.

The present study is concerned with effects of ginseng diols and triols on the tone of aorta and the interaction of Ca^{++} with the cellular components associated with the excitation-contraction

processes. The rational lies on the basis that the mobilization of Ca^{++} upon the excitation of muscle cell determines the degree of contraction. And the Ca^{++} mobilization is, in turn, regulated by cellular components involved with the excitation-coupling processes.

Methods

Aortic strips were prepared from rabbit and the tone of aortic strips was determined in vitro system as illustrated in figure 1. The tone of aorta

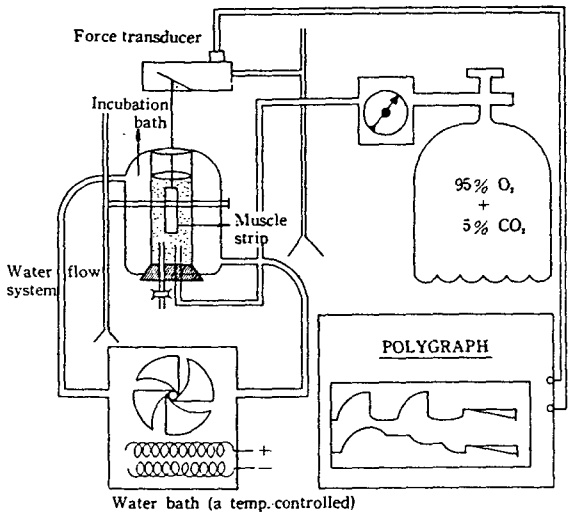


Fig. 1. Schematic diagram of system to record the change of myocardial contractility at polygraph (Grass Model 7).

was maintained by the addition of norepinephrine and the effect of diol or triol on the tone was observed following the administration of these glycosides into the incubation medium. Sarcoplasmic reticulum was prepared from rabbit hearts by the method described in table 1 and the active Ca^{++} uptake was measured by the Milipore technique employing Ca^{45} in the medium shown in Table 2.

Sarcolemma was prepared from rabbit hearts by the method described in Table 3 and the Ca^{++} binding was measured according to the method of Kang and Lee(1978) employing the Corning glass cover method.

Table 1. Flow sheet diagram for preparation of sarcoplasmic reticulum

Chopped ventricular muscle + 3 volume of
0.3 M sucrose
2 mM ascorbic acid
20 mM Tris-HCl (pH 7.2)
↓
Homogenize for 15 sec with Vir Tis Homogenized
add 10 ml of homogenizing solution
↓
Centrifuge for 20 min at 12,000 x g
↓
sediment discard | supernatant
↓
centrifuge for 90 min at 40,000 x g
↓
pellet (sarcoplasmic reticulum) suspend in
0.3 M sucrose
1 mM potassium oxalate.
5 mM Tris-histidine (pH 7.2)
| supernatant discard

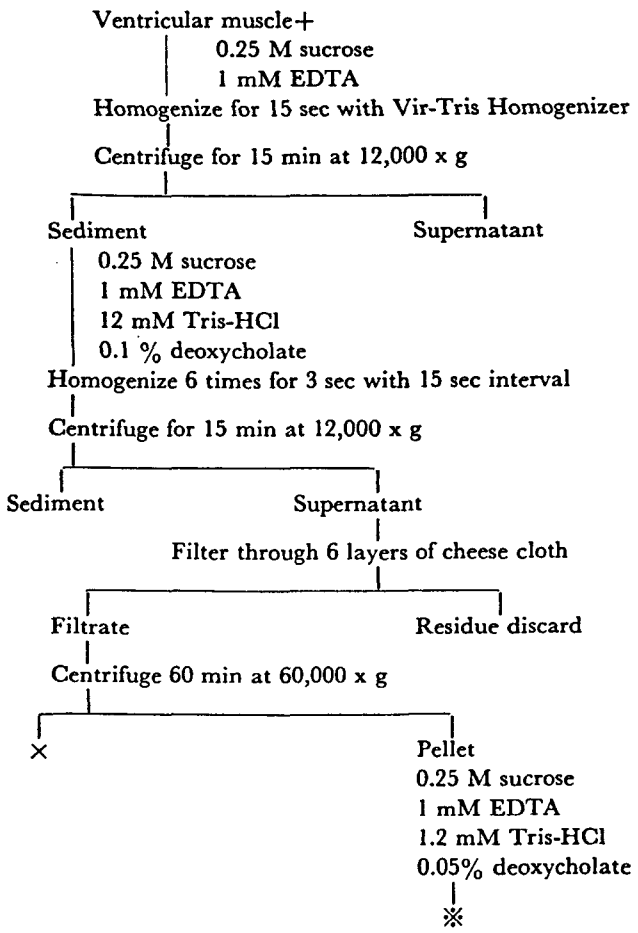
Table 2. Radioisotope Ca^{45} uptake by SR

100 mM Tris-histidine (pH 7.2)		1 ml
1 M KCl		1 ml
50 mM $MgCl_2$		1 ml
0.3 mM $CaCl_2$		1 ml
50 mM Sod. oxalate		1 ml
50 mM ATP		1 ml
100 mM CP		1 ml
1 mg/ ml CPK		1 ml
1.0 mg/ml Protein		1 ml
water		1 ml
Ca^{45} 0.05 ml of stock (2 μci)	Total	10 ml

Results

1. The effect of diol and triol on the aortic strip is shown in Tables 4 and 5 and figures 2 and 3. The summary of results are shown in figure 4. As can be seen in these figures, both diol and Triol exhibit the relaxing effect on the aorta and triol has an effect stronger than that of diol.
2. The effect of glycosides on the Ca^{++} uptake of sarcoplasmic reticulum is shown in figures 6 and 5. As can be seen in these figures, both glycosides have an inhibitory effect on the Ca^{++} uptake of sarcoplasmic reticulum in the presence of ATP. However, triol has a more potent inhibitory effect on the Ca^{++} uptake than diol.

Table 3 (1) Flow sheet diagram for preparation of membrane



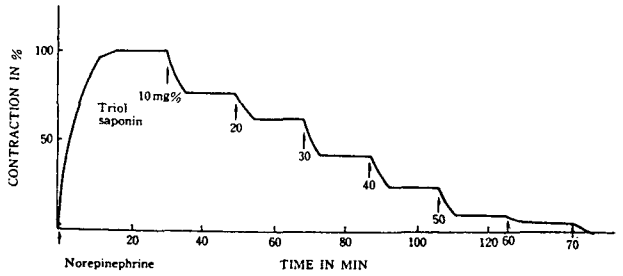


Fig. 2.

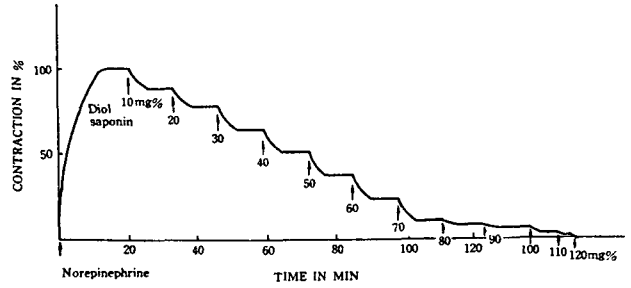


Fig. 3.

Table 3 (2)

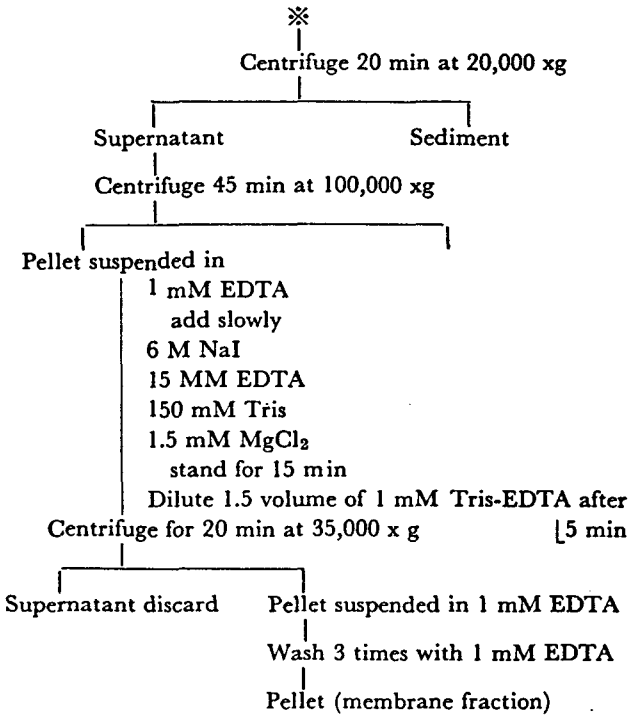


Table 4. Effect of triol saponin on the norepinephrine Induced Contraction of Descending Aortic Strip

Dose (mg%)	10	20	30	40	50	60	70
Relax (%)							
Mean	23.1	36.6	58.2	75.4	90.5	94.4	100

3. The effect of glycosides on the Ca^{++} binding characteristics of cellular membrane is shown in table 6. As this table shows both glycosides inhibit the Ca^{++} binding to the cellular membrane. However, the effect of triol is more potent than that of diol.

Discussion

The present concept of excitation-contraction coupling is as follows. Upon the arrival of excitation wave along the cellular membrane, Ca^{++} bound to the cellular membrane and sarcoplasmic reticulum is released and increased the intracellular Ca^{++} concentration. The amount of Ca^{++} thus released determines the degree of contraction of muscle. The present study indicate that Ginseng glycosides inhibit the Ca^{++} binding to sarcoplasmic

Table 5. Effect of diol saponin on the norepinephrine induced contraction of descending aortic strip.

Dose (mg%)	10	20	30	40	50	60	70	80	90	100	110	120
Relax (%)												
Mean	10.7	20.9	34.7	49.0	62.1	76.2	88.7	91.2	93.3	96.9	98.3	100

Table 6. Effect of triol on calcium binding of cell membrane

Concentration of Triol	0	20 mg%	40 mg%	60 mg%	80 mg%
Radioactivity CPM/mg protein	2497 ±101.6	2511 ±106	2394 ±96	1910 ±94	1781 ±102

Incubation medium: 20 mM Tris-maleate (pH 7.4). 3 mM ATP (Tris) 3 mM $MgCl_2$, 1 mM $CaCl_2$, 3 μ Ci/ml ^{45}Ca, $Nacl_2$ 100 mM.

Table 7. Effect of diol on calcium binding of cell membrane

Concentration of Diol	0	20 mg%	40 mg%	60 mg%	80 mg%
Radioactivity CPM/mg protein	2541	2610	2491	2388	2088
CPM/mg protein	±112	±101	±98	±106	±97

Incubation medium: same as Table 6.

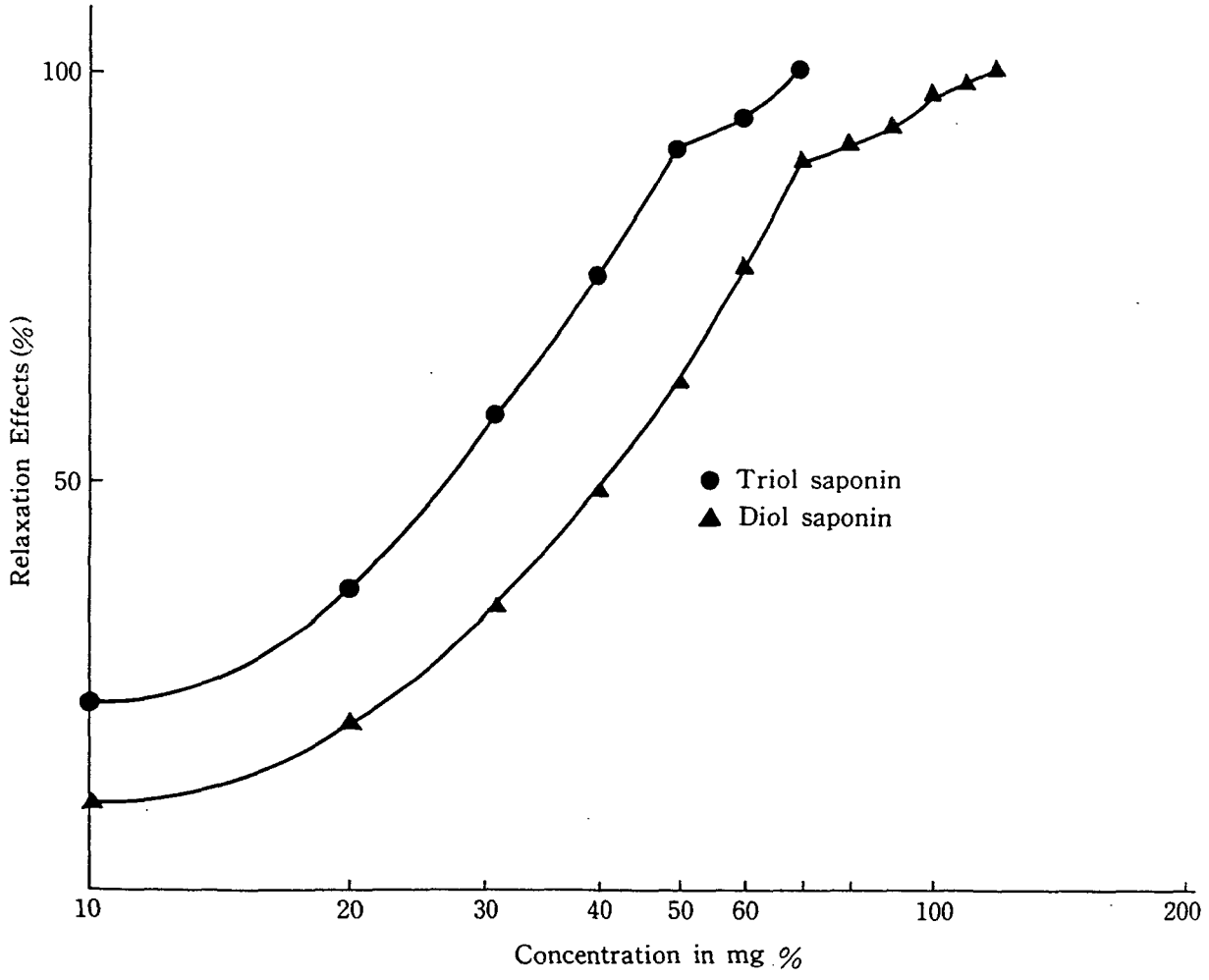


Fig. 4. Effect on aortic strip relaxation

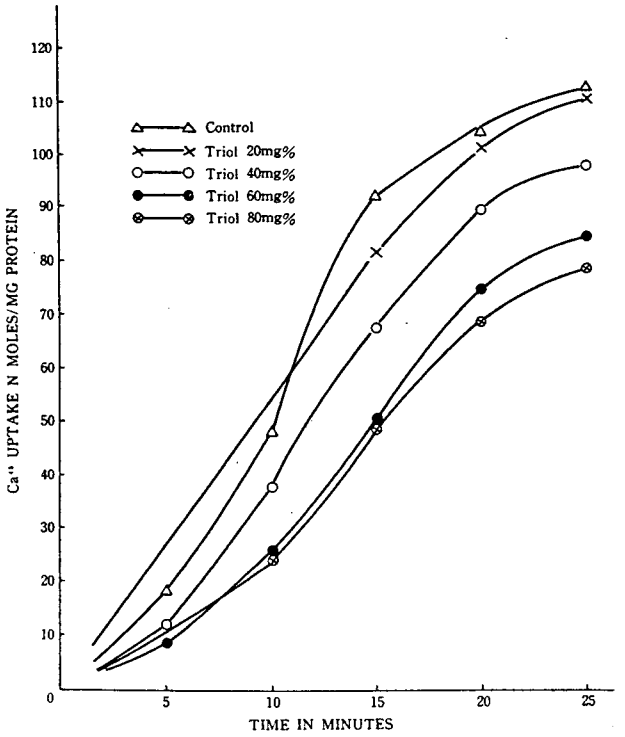


Fig. 5. Triol effect

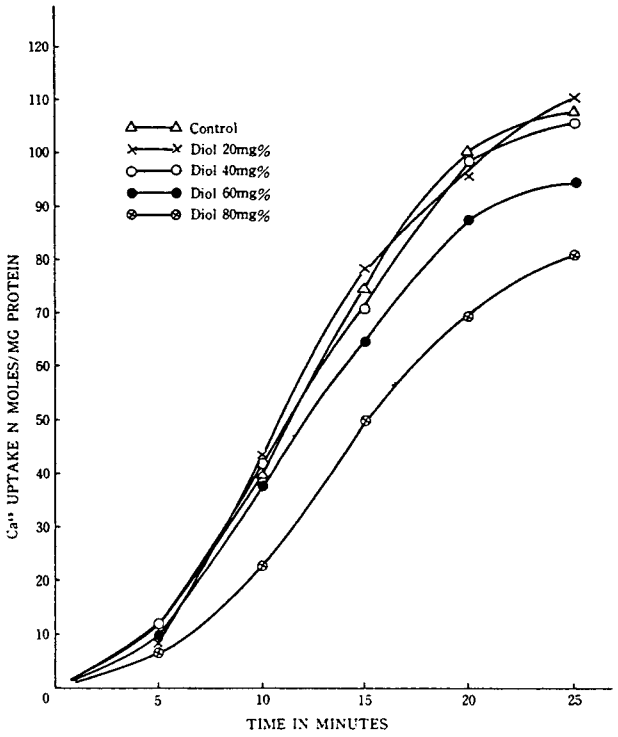


Fig. 6. Diol effect

reticulum and cellular membrane. This would result in the decreased amount of Ca^{++} bound to the intracellular membrane. Consequently, the presence of Ginseng glycosides would decrease the amount of Ca^{++} released upon excitation of smooth muscle cell. This conclusion is consistent with the results obtained in the aortic strip. The glycosides were found to decrease the aortic tone. This pharmacological effect is more prominent with triol than diol. Thus it is concluded that Ginseng releaves the hypertension by relaxing the smooth muscle of blood vessels. The cellular mechanism of this relaxing effect appear to reside with the ability of glycosides to decrease the Ca^{++} binding to the cellular components involved with the excitation coupling processes.

Chairman: Now the time is open to discussion.

Fulder: I want to question the physical significance of these results. Experiments were very carefully done but I don't quite understand whether any saponin would do this. You used quite high doses, unphysiological doses, because it is in vitro experiment and saponins are surface active. So, did you do some controls to find out whether any saponin would do this or specific effect of ginseng saponins?

Lee: Well, orginally we have been interested in glycosides and usually these saponins, if we use a large amount matter but usually they don't do it. And this amount of glycosides for instance if you take an example of cardiac glycosides, which is very potent cardiac glycoside as far as sodium uptake is concerned, doesn't have any effect on the celluler membrane of calcium binding. Therefore, this ginseng inhibition cannot be attributed as a nonspecific one, where some specificity is involved in this. However, on the other hand, the quantitative relationship of this in vitro study with in vivo is quite a speculation here. So, I don't mean that this will be the one mechanism which will induce hypotensive effect. This is another possibility we presented as there is, but as far as quantitative relationship is concerned, we have no confidence whatsoever.

Yamamoto: Could you teach us about the pos-

sible role of psychonuclear or ginseng saponin on vascular muscle?

Lee: I wouldn't try to teach you because you know more than I do probably, but I will try to explain what I think. Well, actually the cyclic AMP role on metabolism, especially in cardiac muscle has been quite widely studied these days and apparently there is definite involvement of cyclic AMP in the SR fraction. As you know cyclic AMP is supposed to be mostly cellular membrane and not intra- cellular. And yet, apparently there are component involved intracellularly and SR. This has been done by Cincinati group and also by some group on this cyclic AMP influence on the SR uptake and they were studying this one in particular with refence to effect of catecholamine because as you know catecholamine has very potent cardio effect. They like to attribute this cardio effect of cyclic catecholamine on this contractility and you brought this in SR function. And naturally if you can connect something with calcium uptake influence on the calcium uptake SR, then you can explain some of the catecholamine effect on the heart. They studied quite a bit on this and they have some positive result. But at present it is very hard to pinpoint clearly where and how this cycle AMP is involved between intracellular component, extracellular membrane. This is very clearly shown. But intracellular we are not quite sure. This is under investigation at the present time.

Shibata: I'd like to make clear that "did you use the saponin or sapogenin in your test you mentioned?" Did you use panaxadiol or panaxatriol? This is a sapogenin and which do you use?

Lee: Dr. Shibata, I thought that you would ask that I was asked similar things last symposium and this was the thing I was supplied with this preparation by the Ginseng Research Institute here and we did not have any chemical analysis or component. I don't know much about this chemical component of this fractions. It is given to us as a fraction and I have no knowledge on the chemical nature of this fraction.

Shibata: From my experiences in vitro experiment, I like to show much distinct action of the glycosides. So, I wonder which do you use. I'd rather make it clear.

Lee: Thank you much, Dr. Shibata.

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