Br. J. Pharmac. (1986), **88**, 259–267

Positive inotropic action of saponins on isolated atrial and papillary muscles from the guinea-pig

Yoshikazu Enomoto, Katsuaki Ito[1], Yasushi Kawagoe, Yasunori Morio & Yasundo Yamasaki

Department of Veterinary Pharmacology, Faculty of Agriculture, University of Miyazaki, 889–21, Japan.

1 The effects of several saponins of animal and plant origin on the contractile activity of atrial and papillary muscles of the guinea-pig were tested. In a concentration of 1×10^{-5} M, holothurin-A (HL-A), holothurin-B, echinoside-A, echinoside-B and sakuraso-saponin (Saku) exhibited positive inotropic and chronotropic actions whereas desacyl-jego-saponin and ginsenoside-Rd did not.

2 Saponins having a positive inotropic action caused haemolysis of rabbit erythrocytes whereas those without inotropic action did not cause haemolysis.

3 The positive inotropic action of saponins was not affected by practolol, chlorpheniramine, cimetidine and indomethacin.

4 Verapamil (10^{-6} M) inhibited the inotropic actions due to HL-A and isoprenaline (10^{-8} M) to the same extent but had a small efect on those due to ouabain (10^{-7} M).

5 In high K^+ (30 mM K^+) medium where the action potential and the contraction were depressed, HL-A, Saku and isoprenaline restored the action potential and the contraction of the 'slow response' type whereas ouabain failed to do so.

6 In normal medium HL-A and Saku reduced the resting membrane potential by 15–20 mV.

7 These results suggest that modification of the Ca channel is involved in the positive inotropic action of saponins.

Introduction

Some saponins are known to interact with cholesterol in biological membranes and alter the membrane permeability. At high concentrations, saponin produces a pore in the cell membrane as a result of the formation of insoluble complexes with cholesterol (Bangham & Horne, 1962; Glauert *et al.*, 1962; Ohtsuki & Ozawa, 1977; Ohtsuki *et al.*, 1978). For this reason some saponins are used as a tool to make chemically skinned preparations of cardiac muscles and smooth muscles (Endo & Kitazawa, 1978; Saida & Nonomura, 1978). Since it is suggested that cholesterol may influence passive transport, carrier mediated transport or enzymatic activity of membranes (Demel & De Kruyff, 1976), saponin at a 'sub-skinning' concentration may modify mechanical and electrical performance of muscles. The study of the pharmacology of saponins should provide useful information about the role of cholesterol in membrane functions.

In this study we have examined the effects of several saponins on the mechanical and electrical properties of cardiac muscles from guinea-pigs. We used saponins of plant and animal origin, the structures of which had already been determined. The effects of saponins on the contractions were compared with those of isoprenaline and ouabain. Preliminary accounts of this study have appeared elsewhere (Yamasaki *et al.*, 1983; 1984).

[1]Author for correspondence

Methods

Tension experiments

Right and left atrial muscles and papillary muscles of right ventricles were isolated from the hearts of guinea-pigs (Hartley) killed by a blow on the neck. Muscles were mounted in an organ bath containing 10 ml Tyrode solution gassed with 95% O_2 and 5% CO_2 at 36°C with an initial tension of 0.5 g. The contraction was measured isometrically and recorded on a pen-writing oscillograph. Left atrial and papillary

muscles were stimulated via bipolar platinum electrodes with a square wave pulse of 1 ms duration at a frequency of 1 Hz. Tyrode solution had the following composition (mM): NaCl 136.8, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 0.05, $NaHCO_3$ 11.9 and dextrose 5.5 (pH 7.3–7.4). High K^+-Tyrode solution was prepared by adding 25 mM KCl to the above solution.

Electrophysiological experiments

The papillary muscle was placed horizontally in a bath in which 20 ml Tyrode solution circulated with the aid of 95% O_2 and 5% CO_2. One end of the muscle was connected to a force-displacement transducer to record the tension. A cell was impaled on a microelec-

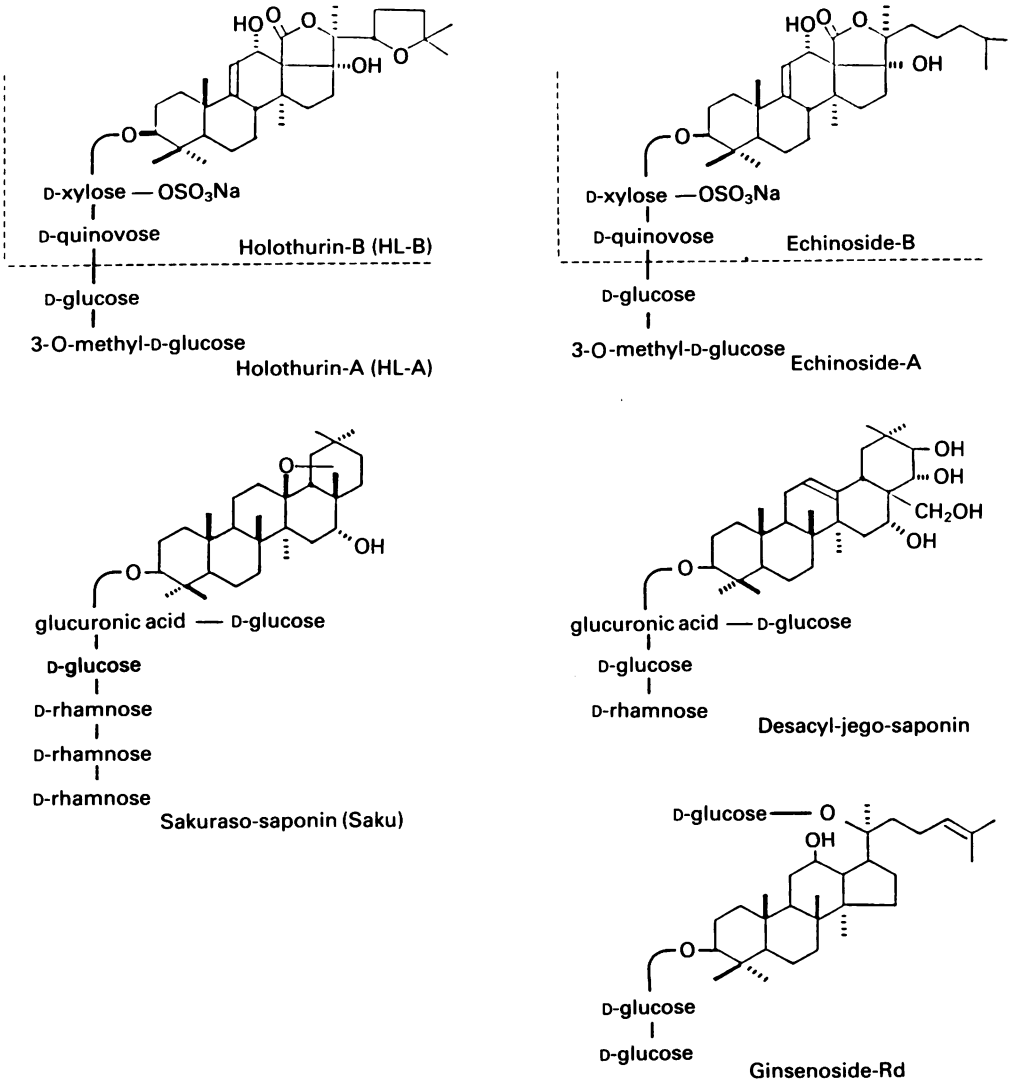

Figure 1 Structures of saponins used. The structure of holothurin-A was determined by ^{13}C n.m.r. (Kitagawa *et al.*, 1979). The structure was inconsistent with that reported by Chanley *et al.* (1959), who assumed the sequence of the monosaccharide to be quinovose, 3-O-methyl-glucose, glucose and xylose from the terminal.

trode filled with 3 M KCl and having a tip resistance between 8 to 15 MΩ and the transmembrane potential was displayed on a cathode ray oscillograph. The contraction and the potential were recorded on a chart or photographed. The muscle was stimulated at a frequency of 1 Hz in normal Tyrode solution and 0.1 Hz in high K-Tyrode solution.

Haemolysis experiments

Heparinized blood from rabbits was collected in physiological saline solution (PSS) consisting of (mM): NaCl 154.0, NaH_2PO_4 1.6, Na_2HPO_4 9.6 (pH 7.4). The red blood cells were rinsed three times with PSS by centrifugation at 3000 r.p.m. for 10 min. The packed cells were suspended in PSS to give a haematocrit value of 8 ± 1%. Each saponin in a volume of 0.2 ml was added to a 3 ml suspension of erythrocytes in PSS. Three aliquots were used for each concentration. The solution was incubated for 1 h at 37°C and then centrifuged for 3 min at 3000 r.p.m. The absorbance at 543 nm of the supernatant was measured with a spectrophotometer. The 100% haemolysis was achieved by adding erythrocytes in distilled water and zero % haemolysis was obtained from the suspension without saponins. Percentage haemolysis was calculated from these values. The slope for concentration-response curve and the concentration causing 50% haemolysis were calculated from the regression line of % haemolysis on log concentration of saponin.

Drugs

Saponins used were holothurin-A (HL-A), holothurin-B (HL-B), obtained from a sea cucumber *Holothuria leucospilota*, echinoside-A, echinoside-B, obtained from another sea cucumber *Actinopyga echinites*, sakuraso-saponin (Saku), obtained from a primrose *Primula sieboldi*, desacyl-jego-saponin from a storax *Styrax japonica* and ginsenoside-Rd from a ginseng *Panax ginseng*. The structures of these saponins are shown in Figure 1. HL-A, echinoside A, Saku and desacyl-jego-saponin were dissolved in distilled water at a concentration of 1 mM. HL-B, echinoside-B and ginsenoside-Rd were dissolved in 0.1% dimethyl sulphoxide in the same concentration. We made sure

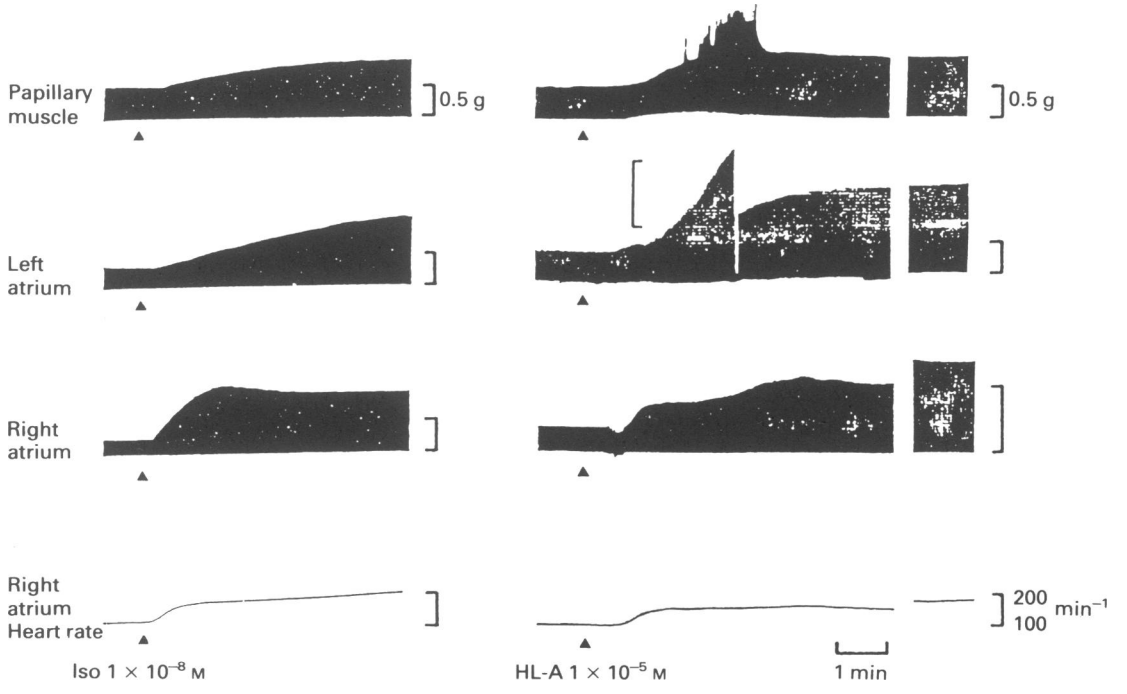

Figure 2 Typical records of effects of isoprenaline (Iso) 1×10^{-8} M and holothurin-A (HL-A) 1×10^{-5} M on the contractions of the guinea-pig papillary, left and right atrial muscles and the heart rate of the right atrial muscle. The papillary muscle and the left atrium were driven at 1 Hz. After washing out Iso, HL-A was applied. HL-A caused arrhythmia during the development of the positive intropy in the papillary muscle (upper, right). The sensitivity of the recorder was changed during the positive inotropy due to HL-A in the left atrium (middle, right).

that dimethyl sulphoxide did not influence the mechanical and electrical properties of the muscles when it was diluted in the bath. We could not use saponins in a concentration above 1×10^{-4} M because foam caused by saponins in the bath disturbed the measurements. Other drugs used were isoprenaline (Sigma), ouabain (Merck), chlorpheniramine (Sankyo), cimetidine (SK&F), verapamil (Knoll-Eisai), practolol (ICI) and indomethacin (Sigma).

Statistics

Data are expressed as mean ± s.e. with the exception of the data from haemolysis experiments which are expressed as the mean value of three aliquots. Differences were considered to be significant when $P < 0.05$.

Results

Effects of saponins on the contractile force of cardiac muscles of guinea-pigs

The left atria and papillary muscles of the guinea-pigs were driven at a frequency of 1 Hz for 30 min as an equilibration period while the right atria were allowed to beat spontaneously during the period. At first isoprenaline 1×10^{-8} M was administered to check the responsiveness of the muscle to inotropic agents. Thereafter saponin in a concentration of 1×10^{-5} M was applied to the muscle. The typical records of the effect of isoprenaline and HL-A on the contraction of muscles are shown in Figure 2. HL-A, HL-B, echinoside-A, echinoside-B and Saku had a positive inotropic effect on the muscles whereas desacyl-jego-saponin and ginsenoside-Rd had negligible effect (Table 1). Saponins having an inotropic action also increased the rate of spontaneous contraction in the right atria. Although we did not test the dose-dependency of the effects of saponins except HL-A, at a concentration of 1×10^{-5} M, the inotropic effect of HL-A was most potent followed by Saku and HL-B.

Table 2 summarizes the effects of varied doses of HL-A and isoprenaline on the twitch tension and the time to the maximum effect in the left atrial and papillary muscles. The maximum effects were measured when the muscles contracted at a frequency of 1 Hz. Both substances dose-dependently augmented the contractile force. The time to the maximum effect due to isoprenaline was relatively independent of the dose while that due to HL-A was shortened in a dose-dependent manner. When higher concentrations of saponins were applied, extrasystolic contractions often appeared during the development of the positive inotropic action (for example, papillary muscle in Figure 2) and the diastolic tension was increased at the later stage in many preparations.

Effects of various drugs on the positive inotropic action of saponins

The positive inotropic and chronotropic actions of saponins were not affected by practolol 1×10^{-5} M, chlorpheniramine 1×10^{-6} M, cimetidine 1×10^{-5} M and indomethacin 1×10^{-6} M. On the other hand, practolol 1×10^{-5} M completely inhibited the positive inotropic action due to isoprenaline 1×10^{-8} M. Therefore, it is concluded that endogenous catecholamine, histamine and prostaglandins are not involved in the positive inotropic and chronotropic actions of saponins.

The influence of an organic Ca channel blocker, verapamil, on the saponin-induced positive inotropic action was tested in comparison with that on isoprenaline- and ouabain-actions. Papillary and left atrial muscles drive at 1 Hz were pretreated with verapamil (1×10^{-6} M) for 40 min, which decreased the twitch tension to 68 ± 4% and 65 ± 5% of the predrug level, respectively. The positive inotropic action of HL-A,

Table 1 Effects of various saponins (10^{-5} M) on the contractile force of papillary muscles, left and right atria of guinea-pigs and the heart rate of the right atria

Saponins	*Contractile force* *Papillary muscles*	*Left atria*	*Right atria*	*Heart rate* *Right atria*
HL-A	159 ± 35	256 ± 42	138 ± 30	42 ± 9
HL-B	148 ± 17	64 ± 23	100 ± 24	35 ± 9
Echinoside-A	108 ± 27	118 ± 22	120 ± 26	42 ± 8
Echinoside-B	34 ± 11	63 ± 18	78 ± 43	17 ± 3
Saku	140 ± 23	180 ± 24	108 ± 19	62 ± 20
Desacyl-jego-saponin	0 ± 0	0 ± 0	3 ± 0	0 ± 0
Ginsenoside-Rd	2 ± 2	12 ± 5	2 ± 2	0 ± 0

Values are expressed as mean ± s.e. of the maximum % increase from the predrug level; $n = 6-11$. Contractile force was measured when the muscle did not exhibit extrasytolic contraction. HL-A = holothurin-A; HL-B = holothurin B.

Table 2 Dose-dependent effects of holothurin-A (HL-A) and isoprenaline on the contractile force and the time to the maximum inotropy in papillary muscles and left atria

Dose	*Contractile force (%)*		*Time to maximum inotropy (min)*	
	Papillary muscles	*Left atria*	*Papillary muscles*	*Left atria*
HL-A				
3×10^{-6} M	89 ± 15	165 ± 73	27.4 ± 3.5	28.1 ± 1.9
1×10^{-5} M	159 ± 35	256 ± 42	6.0 ± 0.6	7.8 ± 0.8
3×10^{-5} M	475 ± 74	273 ± 52	4.6 ± 0.5	2.9 ± 0.2
Isoprenaline				
1×10^{-8} M	67 ± 18	77 ± 34	6.8 ± 0.7	7.5 ± 0.3
3×10^{-8} M	161 ± 13	194 ± 25	6.3 ± 1.3	6.4 ± 1.5
1×10^{-7} M	239 ± 68	382 ± 90	5.7 ± 0.9	6.3 ± 0.5

Values are expressed as mean ± s.e. $n = 6$–8. Percentage increase of the contractile force represents its maximum effect.

isoprenaline or ouabain was significantly suppressed by verapamil as summarized in Table 3. Of the three, ouabain was most resistant to verapamil. Next, the effects of La^{3+} on the HL-A-, isoprenaline- and ouabain-induced positive inotropic actions were compared. In these experiments the Tyrode solution was buffered with Tris hydrochloride instead of sodium bicarbonate. As control in this solution, HL-A (10^{-5} M), isoprenaline (10^{-8} M) and ouabain (10^{-7} M) augmented the contractile force of the papillary muscles by 143 ± 12%, 53 ± 13% and 188 ± 44%, respectively, and that of the left atria by 270 ± 45, 43 ± 6 and 314 ± 74%, respectively. The inhibitory actions of La^{3+} on the positive inotropy due to each substance are also shown in Table 3. In contrast to verapamil, La^{3+} suppressed the inotropic action of HL-A, isoprenaline and ouabain to a similar extent.

Restoration of contraction in K^+-depolarized papillary muscles by saponins

In this series of experiments the effect of HL-A on the mechanical activity of the K^+-depolarized papillary muscles was examined in order to assess whether an effect on the Ca channel is involved in the positive inotropic action of saponins. When the external K concentration was increased to 30 mM, the muscles could not respond to stimulation at 1 Hz but responded to 0.1 Hz by very small contractions. While driving the muscle at a frequency of 0.1 Hz, a cumulative increase of the external Ca^{2+} concentration enhanced the contraction. The presence of HL-A (1×10^{-5} M) or isoprenaline (1×10^{-8} M) potentiated the contraction of partially depolarized muscles as shown in Figure 3. In contrast, ouabain did not affect the extracellular Ca concentration-twitch relationship of the muscles (Figure 3).

Changes in transmembrane potential of papillary muscles due to saponins

Both HL-A and Saku reduced the resting membrane potential, the action potential amplitude of the papillary muscle, and shortened the action potential duration. Figure 4 shows an example of changes in the action potential and the contraction induced by HL-A 1×10^{-5} M. The depolarization due to HL-A and Saku was biphasic, i.e., at first the resting membrane potential was reduced to −60 t0 −65 mV, then repolarized to −70 to −75 mV and maintained this level. Although the steady state depolarization was the same with HL-A and Saku, the development of depolarization was considerably faster with HL-A than Saku, the first peak of depolarization due to HL-A appearing at 3 min whereas that due to Saku appeared at 10 min. Under these experimental conditions, on the whole, the muscle seemed to be more sensitive to saponins than in the experiments shown in Figure 2 because the diastolic tension developed rapid-

Table 3 Inhibition by verapamil 10^{-6} M and La^{3+} 5×10^{-4} of positive inotropic actions due to holothurin-A (HL-A, 10^{-5} M), isoprenaline (10^{-8} M) and ouabain (10^{-7} M)

Drugs	*Papillary muscles*		*Left atria*	
	Verapamil	*La*	*Verapamil*	*La*
HL-A	55 ± 15	84 ± 2	58 ± 7	51 ± 7
Isoprenaline	49 ± 15	71 ± 5	42 ± 18	62 ± 12
Ouabain	38 ± 4*	73 ± 4	10 ± 8*	71 ± 7

Data are expressed as % inhibition of the positive inotropic action due to each substance. $n = 6$–10.
*Significantly different from HL-A.

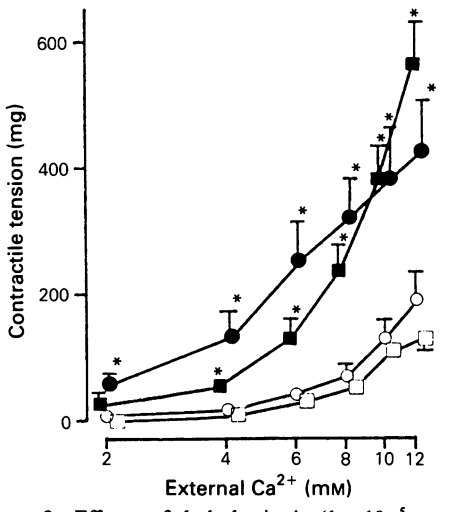

Figure 3 Effects of holothurin-A (1×10^{-5} M, ●), isoprenaline (1×10^{-8} M, ■) and ouabain (1×10^{-7} M, □) on the contraction of the papillary muscles partially depolarized by 30 mM KCl. (○) Control. The external Ca concentration was cumulatively increased in the presence of inotropic agents. Each point represents mean of 6–9 preparations with s.e. shown by vertical lines. *Significantly different from control ($P < 0.05$).

ly after 1×10^{-5} M HL-A and Saku in this experiment. The positive inotropic action was prominent during the first depolarization phase.

Next, HL-A and Saku were applied to the muscles which had been partially depolarized by 30 mM KCl which reduced the resting potential to about −40 mV and abolished the action potential and the twitch. After treatment with HL-A or Saku, stimulation at a frequency of 0.1 Hz induced the slow action potential and contraction as shown in Figure 5. The threshold concentration required to induce the slow action potential was the same for HL-A and Saku, that is, 3×10^{-5} M. Isoprenaline at 1×10^{-8} M also restored the slow action potential and the contraction while ouabain 1×10^{-6} M had no effect. When external NaCl was replaced with iso-osmolar choline chloride, stimulation failed to evoke an action potential and a contraction. In these circumstances, HL-A restored the action potential and the contraction although the effect was temporary.

Haemolytic activity of saponins

Concentrations of saponins that induced 50% haemolysis of rabbit erythrocytes and the slope of the log concentration-response curve for haemolytic

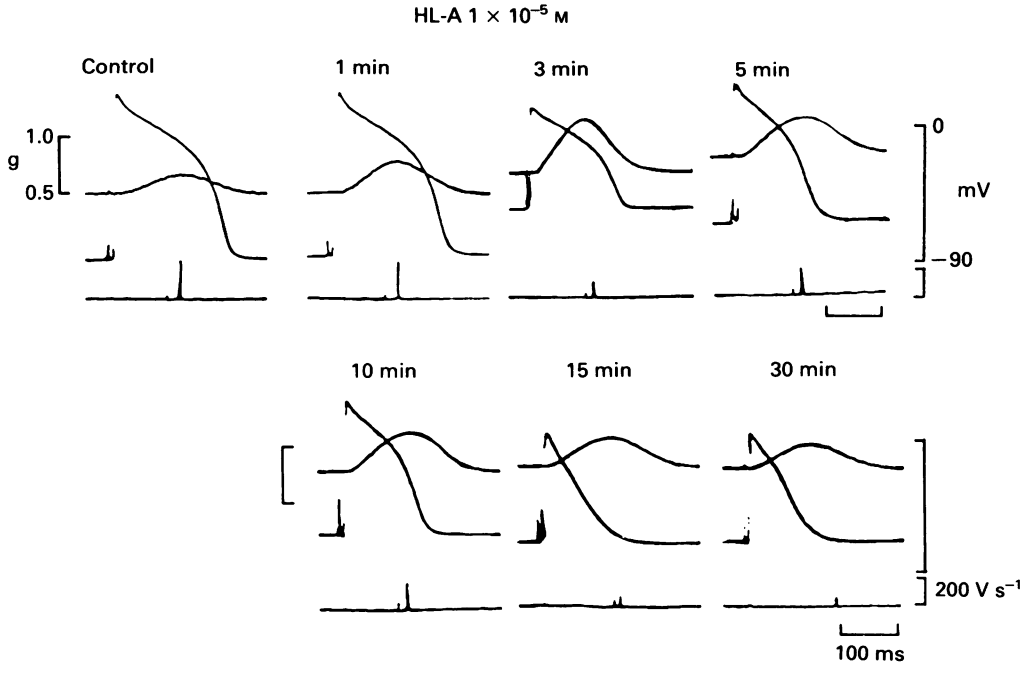

Figure 4 Changes in the contraction (the upper trace), the action potential (the middle trace) and the maximum rate of rise of action potential (the bottom trace) in guinea-pig papillary muscle during exposure to holothurin-A 1×10^{-5} M (HL-A). The muscle was driven at a frequency of 1 Hz. A signal of the maximum rate of rise was displayed on an oscillograph with some delay to avoid the overlap on the trace of action potential.

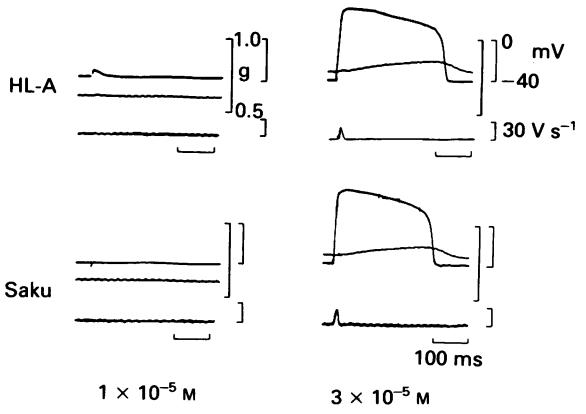

Figure 5 Restoration of the action potential and the contraction of partially depolarized papillary muscles following application of holothurin-A (HL-A) and Saku. In the right panels, trace with a large amplitude: action potential; slowly rising trace: contraction and bottom trace: maximum rate of rise of action potential. High potassium (30 mM) inactivated the action potential and the contraction. The threshold concentration required to induce the slow response was 3×10^{-5} M for both HL-A and Saku.

activity are summarized in Table 4. Although the curves did not parallel each other, it is apparent that echinoside-A was most potent followed by HL-A. The haemolytic activities of Saku and HL-B were less than those due to echinoside-A and HL-A since the concentration-response curves of Saku and HL-B were on the right of the curve of HL-A. The slope of the curve for echinoside-B was much less than those for other saponins having haemolytic activity. On the other hand, desacyl-jego-saponin and ginsenoside-Rd, which lacked inotropic action, were without haemolytic activity.

Discussion

Saponin, at a concentration of 50 μg ml^{-1} is used for chemical skinning of cardiac muscles (Endo & Kitazawa, 1978). If skinning of the membrane is achieved, the cell allows the entry of any ion or even a macromolecule (Ohtsuki & Ozawa, 1977; Ohtsuki *et al.*, 1978) so that the membrane potential should be completely abolished. The concentrations of saponins used in this study were between 1.8 μg and 36 μg ml^{-1}. Although the concentration tested was close to that required for chemical skinning the saponins did not skin the muscle membranes in our experiments because the magnitude of the saponin-induced depolarization in a cell located on the surface of the tissue was only about 20 mV. The lack of skinning action of saponins in this study may be due to the different experimental conditions and the different types of saponin used.

We demonstrated that saponins had a positive inotropic action in cardiac muscles. This action was not mediated by endogenous substances, such as catecholamines, histamine or prostaglandins. Every saponin which had haemolytic activity exhibited a positive inotropic action. Therefore we can say that the positive inotropic action was common for saponins having an action on membrane permeability. Similarly, Nakamura *et al.* (1979) found that only saponin which had haemolytic activity increased the permeability of liposomes. Probably a target of the saponin-inotropy was the plasmalemma but not the membrane of the sarcoplasmic reticulum since saponin had little effect on Ca^{2+} uptake by the sarcoplasmic reticulum, which is devoid of cholesterol (Inamitsu & Ohtsuki, 1984; Kwan & Lee, 1984).

We compared the mode of inotropic action of HL-A, the most potent cardiotonic saponin tested, with that of isoprenaline and ouabain. The action of HL-A resembled that of isoprenaline rather than ouabain in

Table 4 Haemolytic activity of saponins in rabbit erythrocytes

Saponin	*Concentration to cause 50% haemolysis* (M)	*Slope of the regression line*
HL-A	2.1×10^{-6}	218
HL-B	6.4×10^{-6}	94
Echinoside-A	1.6×10^{-7}	131
Echinoside-B	4.5×10^{-6}	30
Saku	3.6×10^{-6}	96
Desacyl-jego-saponin	—	0
Ginsenoside-Rd	—	0

Slope was calculated from the regression line of % haemolysis on log saponin concentration. HL-A = holothurin-A; HL-B = holothurin B.

some respects. First, the actions of HL-A and isoprenaline exhibited similar sensitivity to verapamil whereas the action of ouabain was less sensitive to it. It is well known that the action of cardiac glycosides on contraction or ^{45}Ca uptake of cardiac muscles is relatively insensitive to organic Ca channel blockers (McCans *et al.*, 1974; Watanabe & Besch, 1974; Burt & Langer, 1982). On the other hand, La^{3+} equally suppressed the actions of HL-A, isoprenaline and ouabain. This is reasonable because La^{3+} decreases any Ca movement across the membrane (Sanborn & Langer, 1970; Langer & Frank, 1972; Reeves & Sutko, 1979; Burt & Langer, 1982). Second, both HL-A and isoprenaline augmented the contraction of partially depolarized muscles depending on the external Ca concentration while ouabain failed to do so. Furthermore, HL-A, Saku and isoprenaline restored the slow action potential of the depolarized muscles. The slow response or the slow action potential can be generated by an activation of the Ca channel in cardiac muscles (Pappano, 1970; Thyrum, 1974; Watanabe & Besch, 1974; Sperelakis & Schneider, 1976). The lack of effect of ouabain on the slow action potential was consistent with data reported by Thyrum (1974) and Watanabe & Besch (1974). Therefore we can say that like isoprenaline, saponin makes the Ca channel available for voltage activation and that this action is at least partly responsible for the positive inotropic action.

Saponins caused sustained depolarization in cardiac muscles as well as in squid axons (De Groof & Narahashi, 1976) or uterine smooth muscles (Osa & Ogasawara, 1984). De Groof & Narahashi (1976) demonstrated that HL-A nonselectively increased ionic permability of squid axon membranes and that, when the depolarization caused by HL-A was complete, the membrane potential did not recover by any ion substitution. We have also found that a specific ionic channel was not involved in the depolarization caused by HL-A or Saku (unpublished data). Therefore the depolarizing action of saponins seems to be related to its pore-forming (skinning) action. Probably, when saponin increases the membrane permeability so as to abolish the membrane potential, skinning of the membrane will occur.

An important question is whether the 'skinning' action of saponin is directly related to the inotropic action. The fact that only saponin at a concentration having haemolytic activity exerted a positive inotropic action suggests that perturbation of the membrane caused by saponin is related to both the actions. However, the order of potency in inducing inotropic action was not necessarily consistent with that required to increase the membrane permeability as mentioned below. As for haemolytic activity, echinoside-A was most potent followed by HL-A. Saku seemed to be slightly weaker than HL-A. Consistent with this action, the order of potency in enhancing the passive permeability of sarcolemmal vesicles isolated from canine ventricles was echinoside-A, HL-A and Saku (Yamasaki *et al.*, 1984). Furthermore, HL-A depolarized the membrane of papillary muscles more rapidly than Saku. Therefore, it can be said that the ability to enhance the membrane permeability or to cause pores in membranes is greatest with echinoside-A, next with HL-A and weakest with Saku. Nevertheless, the inotropic action of HL-A was greater than that of echinoside-A although the entire dose-response relationship was not examined in this study. The inotropic action of Saku was between that of HL-A and echinoside-A. In addition, the ability to induce a slow response of depolarized muscle was the same for both Saku and HL-A. These results suggest that the inotropic action of saponin cannot simply be explained by its effect on membrane permeability. However, it should be noted that absence of parallelism of concentration-haemolysis curves for saponins tested means that the skinning itself is not a simple process. The relationship between these two actions must be clarified by further experiments.

Although the present experiments suggest that modification of the Ca channel is involved in the positive inotropic action of saponins, it is unclear how saponin interacts with the channel. Since it is suggested that metabolic alterations underlie induction of the slow response (Sperelakis & Schneider, 1976), further biochemical studies are needed to clarify how subskinning concentrations of saponins modify the metabolic function controlling the Ca channel.

We ar very grateful to Drs Isao Kitagawa and Iwao Ohtsuki for purification and donation of saponins.

References

BANGHAM, A.D. & HORNE, R.W. (1962). Action of saponin on biological cell membranes. *Nature*, **196**, 952–953.

BURT, J.M. & LANGER, G.A. (1982). Ca^{++} distribution after Na^+ pump inhibition in cultured neonatal rat myocardial cells. *Circulation Res.*, **51**, 543–550.

CHANLEY, J.D., LEDEEN, R., WAX, J., NIGRELLI, R.F. & SOBOTKA, H. (1959). Holothurin. I. The isolation, properties and sugar components of holothurin A. *J. Am. Chem. Soc.*, **81**, 5180–5183.

DE GROOF, R.C. & NARAHASHI, T. (1976). The effects of holothurin A on the resting membrane potential and conductance of squid axon. *Eur. J. Pharmac.*, **36**, 337–346.

DEMEL, R.A. & DE KRUYFF, B. (1976). The function of

sterols in membranes. *Biochim. biophys. Acta*, **457**, 109–132.

ENDO, M. & KITAZAWA, T. (1978). E-C coupling studies on skinned cardiac fibers. In *Biophysical Aspects of Cardiac Muscle*. ed. Morad, M. pp. 307–327. New York: Academic Press.

GLAUERT, A.M., DINGLE, J.T. & LUCY, J.A. (1962). Action of saponin on biological cell membranes. *Nature*, **196**, 953–955.

INAMITSU, T. & OHTSUKI, I. (1984). Characterization of ATP-dependent Ca^{2+} uptake by canine brain microsomes with saponin. *Eur. J. Biochem.*, **145**, 115–121.

KITAGAWA, I., NISHINO, T. & KYOGOKU, Y. (1979). Structure of holothurin A. A biologically active triterpene-oligoglycoside from the sea cucumber Holothuria leucospilota Brandt. *Tetrahedron Ltt.*, **16**, 1419–1422.

KWAN, C.Y. & LEE, R.M.K.W. (1984). Interaction of saponin with microsomal membrane fraction isolated from the smooth muscle of rat vas deferens. *Mol. Physiol.*, **5**, 105–114.

LANGER, G.A. & FRANK, J.S. (1972). Lanthanum in heart cell culture. Effect on calcium exchange correlated with its localization. *J. cell. Biol.*, **54**, 441–455.

McCANS, J.L., LINDENMAYER, G.E., MUNSON, R.G., EVANS, R.W. & SCHWARTZ, A. (1974). A dissociation of positive staircase (Bowditch) from ouabain-induced positive inotropism: Use of verapamil. *Circulation Res.*, **35**, 439–447.

NAKAMURA, T., INOUE, K., NOJIMA, S., SANKAWA, U., SHOJI, J., KAWASAKI, T. & SHIBATA, S. (1979). Interaction of saponins with red blood cells as well as with the phosphatidylcholine liposomal membranes. *J. Pharmac. Dyn.*, **2**, 374–382.

OHTSUKI, I., MANZI, R.M., PALADE, G.E. & JAMIESON, J.D. (1978). Entry of macromolecular tracers into cells fixed with low concentrations of aldehydes. *Biol. Cell.*, **31**, 119–126.

OHTSUKI, I. & OZAWA, E. (1977). Difference in saponin sensitivity between myotubes and mononucleated cells from chick breast muscle. *Cell Stuctr. Func.*, **2**, 367–370.

OSA, T. & OGASAWARA, T. (1984). Effects of a brief treatment with saponin on the contractile and electrical activities of isolated uterine muscle of pregnant rat. *Jap. J. Physiol.*, **34**, 699–712.

PAPPANO, A.J. (1970). Calcium-dependent action potentials produced by catecholamines in guinea pig atrial muscle fibers depolarized by potassium. *Circulation Res.*, **27**, 379–390.

REEVES, J.P. & SUTKO, J.L. (1979). Sodium-calcium ion exchange in cardiac membrane vesicles. *Proc. natl. Acad. Sci., U.S.A.*, **76**, 590–594.

SAIDA, K. & NONOMURA, Y. (1978). Characteristics of Ca^{2+}- and Mg^{2+}-induced tension development in chemically skinned smooth muscle fibers. *J. gen. Physiol.*, **72**, 1–14.

SANBORN, W.G. & LANGER, G.A. (1970). Specific uncoupling of excitation and contraction in mammalian cardiac tissue by lanthanum. Kinetic studies. *J. gen. Physiol.*, **56**, 191–217.

SPERELAKIS, N. & SCHNEIDER, J.A. (1976). A metabolic control mechanism for calcium ion flux that may protect the ventricular myocardial cell. *Am. J. Cardiol.*, **37**, 1079–1085.

THYRUM, P.T. (1974). Inotropic stimuli and systolic transmembrane calcium flow in depolarized guinea-pig atria. *J. Pharmac. exp. Ther.*, **188**, 166–179.

WATANABE, A.M. & BESCH, H.R., JR. (1974). Cyclic adenosine monphosphate modulation of slow calcium influx channels in guinea-pig hearts. *Circulation Res.*, **35**, 316–324.

YAMASAKI, Y., ITO, K. & ENOMOTO, Y. (1983). The cardiac effects of holothurin A, a saponin isolated from seacucumber. *Jap. J. Pharmac.*, **33**, 275P.

YAMASAKI, Y., SUTKO, J.L., ITO, K. & ENOMOTO, Y. (1984). Effects of saponins on the isolated guinea-pig cardiac muscle and canine cardiac sarcolemmal vesicles. *Jap. J. Pharmac.*, **36**, 273P.

(Received June 4, 1985.
Revised October 30, 1985.
Accepted January 3, 1986.)