J.Sci.Soc.Thailand, 21(1995) 11-26

SOME PHARMACOLOGICAL STUDIES OF ARDISIACRISPIN B, AN UTERO-CONTRACTING SAPONIN, ISOLATED FROM *ARDISIA CRISPA*

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(Received November 11, 1994)

ABSTRACT

The present study aimed to characterize the pharmacologic action of the ardisiacrispin B. Studies were performed on in vitro *preparations of uterine smooth muscle, small intestine and thoracic aorta (vascular smooth muscle) obtained from female rats in estrus. Dose- response curves (DR-curve) to ardisiacrispin B, prostaglandin E_2 derivative (Nalador), oxytocin and acetylcholine chloride were obtained. The possible involvement of prostaglandin synthesis in the utero-contracting activity of ardisiacrispin B was explored by investigation of the DR-curve to ardisiacrispin B in the presence of 10^{-6} M indomethacin, a cyclo-oxygenase inhibitor. The local effects of the compound on uterine contractility and cervix softening were also studied* in situ *and* in vitro *respectively.*

Ardisiacrispin B caused dose-dependent contraction of uterine smooth muscle, small intestine and thoracic aortae in a similar pattern to prostaglandin E_2 derivative. Oxytocin also caused uterine strip contraction but had no effect on small intestine. Acetylcholine caused uterine and small intestine contraction in a different manner from that obtained with ardisiacrispin B. However, the presence of indomethacin did not alter the DR-curve to ardisiacrispin B of uterine smooth muscle. In the in situ *experiments, intra-uterine injections of ardisiacrispin B caused uterine contraction in a dose-dependent manner similar to those obtained from prostaglandin E_2 with no changes in mean arterial blood pressure, except that the highest concentration of ardisiacrispin B (6mg/ml) caused lowering blood pressure in some animals. There were no signs of cervix softening after intra-uterine administration of either ardisiacrispin B or prostaglandin E_2, when compared with intra-uterine injections of saline. These results suggest that ardisiacrispin B may exert a prostaglandin E_2-like effect which may act at the prostaglandin E_2-receptor but not by stimulation or enhancement of prostaglandin synthesis.*

INTRODUCTION

Roots of *Ardisia crispa* (Thunb.) A. DC., are used as a combination with some other plants, in Thai traditional medicine to "wash out dirty blood" in women, who suffer from menstrual pain. In Burma, all parts of a related species, *Ardisia humilis* Vahl, are used to treat menstrual disorders.[1] In 1987, Jansakul *et al.* succeeded in isolating two utero-contracting saponins, namely ardisiacrispin A and B, from *Ardisia crispa*.[2] However, the pharmacologic mechanisms of these compounds have not yet been elucidated.

Clinical uses of utero-contracting compounds, such as oxytocin and prostaglandins, include pregnancy termination. Nevertheless, oxytocin is often ineffective and results in many initial induction failures and high rates of cesarean section[3]. Recently, it become apparent that prostaglandin E_2 is a novel compound for induction of labour, owing to its additional effect on induction of cervix ripening[4-6]. However, the limitations of the compound are its instability and incidence of side effects. Thus, it is possible that ardisiacrispin B, which is a stable compound, may be useful as an alternative drug for pregnancy termination. The present study was designed to characterize the pharmacologic action of ardisiacrispin B, the major component isolated from *Ardisia crispa*. Studies were performed *in vitro* using uterine smooth muscle, small intestine and thoracic aorta (vascular smooth muscle) obtained from female rats in estrus. Dose-response relationships of ardisiacrispin B and other known utero-contracting drugs; prostaglandin E_2 derivative (PGE_2), oxytocin and acetylcholine chloride, were determined. Local effects of the ardisiacrispin B, especially on uterine contractility and cervix softening were also investigated *in situ* and *in vitro* , respectively.

MATERIALS AND METHODS

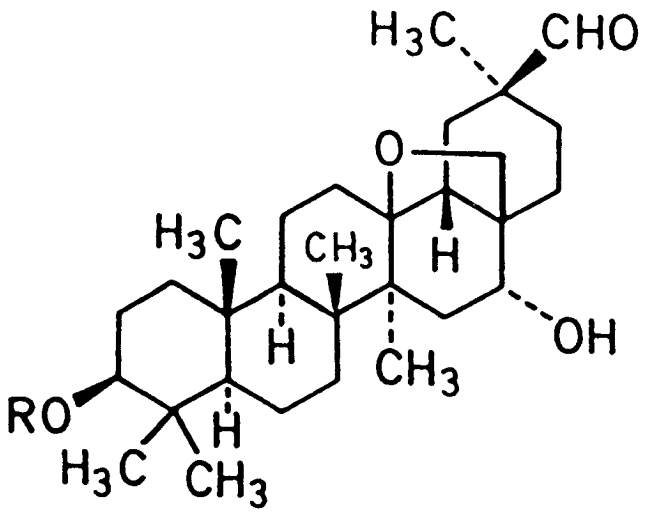
1. Isolation of ardisiacrispin B from *Ardisia crispa*

The plant was identified by Prof. Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University, where a voucher specimen (No. 0000463) has been deposited. Isolation of ardisiacrispin B (ARD_B) followed Jansakul's method[2]. Dried roots of *Ardisia crispa* were ground to a moderately coarse powder and extracted twice with 2% aqueous acetic acid. The combined extracts were concentrated in vacuo and lyophilized. Extract was dissolved in water and the solution shaken three times with water-saturated n-butanol. The butanol phase was evaporated to dryness in *vacuo* and lyophilized. The extract was separated by silica gel column chromatography using water saturated n-butanol as the mobile phase, yielding four different fractions. Only the third fraction contained utero-active component, which was further separated by re-chromatography on a column of silica gel and eluted by a mixture of ethanol:chloroform: water=7:10:3 by volume, and three different fractions were obtained. The utero-active fraction came out first. This fraction was finally purified by preparative high performance liquid chromatography using RP18 column and eluted with a mixture of water: methanol: acetonitrile=6:3:2. The last two fractions contained utero-active compounds of which 20% was ardisiacrispin A and 80% was ardisiacrispin B. Therefore, ardisiacrispin B was chosen for pharmacological study. The chemical structure of ardisiacrispin B is shown in fig.1.

2. Pharmacological studies of ardisiacrispin B

In vitro preparation

Isolated preparations of animal tissues were obtained from female Wistar rats in estus. Animals were killed by cervical dislocation. A uterine sheet approximately 4 mm long was cut from the anterior end of the uterine horn, or a 5 mm length of jejunum removed



Ardisiacrispin B : R = α-L-Rha*p* (1→ 2)-β -D-Glcp(1→ 4)-[β-D-Glc*p*(1→ 2)]-α-L-Ara*p*(1→ 3)

Fig. 1. Chemical structure of ardisiacrispin B.

from the small intestine. Each piece of tissue was mounted in a 20 ml organ bath. The lower end was fixed at the bottom and the other end was connected to a force transducer (Grass FT03). For thoracic aorta, a ring approximately 7 mm long was cut and placed into a 20 ml organ bath using two vertically placed stainless steel stirrup hooks passed through its lumen, the lower being fixed, and the other connected to an isometric force transducer. Contraction were continuously recorded with a Grass Polygraph (7D WU). The organ bath contained Kreb's Henseleit solution of the following composition (mM): NaCl 118.3, KCl 4.7, $CaCl_2$ 1.9, $MgSO_4$ $7H_2O$ 0.45, KH_2PO_4 1.18, $NaHCO_3$ 25.0, glucose 11.66, Na_2EDTA 0.024 and ascorbic acid 0.09, maintained at 37° C, continuously bubbled with a mixture of 95% O_2 and 5%CO_2.

Prior to addition of drugs, tissues were equilibrated for 60 min under a resting tension of 0.5 g for uterus and small intestine and 1.0 g for thoracic aorta. The Kreb's solution was replaced every 10 min.

After equilibration, the contractility of the uterine and small intestine tissue were tested using 10^{-6} M acetylcholine. The presence of functional endothelium of the thoracic aorta was tested as follows. The aortic ring was preconstricted with $3x10^{-7}$ M noradrenaline for 5-8 min (by which time the response had plateaued), and dilator responses to 10^{-6} M acetylcholine recorded. Eighty to ninety per cent vasodilatation to acetylcholine occurred with endothelium-intact rings.

Contractile responses of uterine sheet and small intestine to ardisiacrispin B, PGE_2, oxytocin and acetylcholine.

After testing for the ability of tissue contraction, tissues were incubated for 20 min in Krebs-Henseleit solution. A discrete dose-response relationship for each agonist was obtained, allowing 10-25 min interval between dose. To avoid drug-interaction, each tissue was exposed to only one agonist.

Contractile responses of thoracic aorta to ardisiacrispin B and PGE_2.

After testing for the presence of functional endothelium as described above, tissues were incubated for 45 min in Krebs-Henseleit solution. A cumulative dose-response relationship to PGE_2 was obtained. Following multiple washing to remove PGE_2, the tissues were incubated for 50 min. A cumulative dose-response relationship to ardisiacrispin B was obtained.

Effects of indomethacin on contractile response of uterine sheet to ardisiacrispin B.

After testing for the ability of the contractile activity of the uterine sheet, the tissues were incubated for 40 min in the presence of 10^{-6} M indomethacin. A discrete DR-curve to ardisiacrispin B was obtained.

***In situ* preparation**

Female estrous rats were anesthetized with nembutal (50 mg/kg, i.p.). A polyethylene catheter was introduced through the left common carotid artery and connected to a pressure transducer and polygraph for monitoring blood pressure.

The right abdomen was opened, and a small balloon catheter (filled with 0.9 % normal saline) was introduced through a small incision in the uterus at the cervical end and connected to a pressure transducer and a polygraph for uterine contractility recording. Another catheter was introduced through the ovarian end of the uterine horn for intra-uterine injection of the drugs. The animal was then equilibrated for 1 hr. The response relationships to ardisiacrispin B (0.2-6.0 mg/ml) and to PGE_2 (0.01-0.3 mg/ml) injected intrauterinely, were studied. Allowing 20-35 min intervals between doses. Thus, the uterine lumen (as well as the cervical portion) of each animal was exposed to the drugs for at least two hours. Each concentration of the drug was injected in a volume of 0.05 ml. Time control treatments were also performed by using saline injection instead of any drugs.

***In vitro* preparation for cervix softening studies.**

Cervix softening was studied using a method modified from that of Harkness and Harkness (1959)[7], as follows. At the end of the *in situ* experiments, the animal was killed by overdosage of intraveneous nembutal. The cervical portion of the uterus was removed and mounted onto the 20 ml organ bath using two stainless steel hooks passing through the lumen, the lower being fixed at the bottom, and the other connected to the isometric force transducer, which was mounted on a stand by a micrometer. The organ bath contained Kreb's Henseleit solution, maintained at 37°C, and continuously bubbled with a mixture of 95% O_2 and 5% CO_2. The tissues were equilibrated for 20 min under resting tension of 1.0g. The diameter of the cervix was expanded by 0.5 mm every 1.0 min 5 times (0.5-2.5 mm),

by adjusting the micrometer. The passive tension developed at each stage was recorded. It was expected that, if cervix softening occurred, the passive tension developed at each stage of expansion would be less than that obtained from control animals.

The following drugs were used: prostaglandin E_2 derivative (Nalador, Schering Ltd.,Germany), oxytocin (sigma, U.S.A.), acetylcholine chloride (sigma, U.S.A.) and indomethacin (sigma, U.S.A.). Drugs were dissolved in distilled water except for indomethacin, which was dissolved in 0.1% sodium carbonate (Na_2CO_3) solution. The small balloon catheter (3.0 mm in diameter and 10 mm long with a very thin wall) was made from rubber latex of the same chemical composition as that used for making a surgical gloves.

Statistical analysis

Since the uterine sheet of estrous rats have their own spontaneous contraction, the actions of ardisiacrispin B (ARD_B), PGE_2, oxytocin or acetylcholine chloride (ACh), were expressed qualitatively in terms of increasing rhythmicity or phasic contraction and/or tonic contraction of the tissues, when compared to those obtained before drug administration, and to those obtained from the time treatment controls.

The absoluted tension developed by small intestine, thoracic aorta or cervical tissue in response to the drugs was also measured, so that comparison could be made of maximal contractile responsiveness. The agonist concentration which produced 50% of the maximal response (EC_{50}) was measured by deriving from regression analysis over the linear portion of the DR-curve[8] and expressed as the mean and 95% confidence limits.

Other data are expressed as means±s.e. mean of 4 - 8 experiments (n=4-8), and tests of significance made using student's unpaired t-test or one-way ANOVA. In all cases, a *P* value of 0.05 or less was considered statistically significant.

RESULTS

Fig.2 shows typical contractile responses of the uterine smooth muscle to ARD_B, PGE_2 derivative, oxytocin and acetylcholine, from 4 experiments with each agonist. The contractile responses of these 4 agonists are dose-dependent. The contractile responses to ARD_B of rat uterine smooth muscle are quite similar to those obtained with oxytocin. Small doses of each of these two agonists caused an increase in both amplitude and frequency of phasic contraction compared to basal spontaneous rhythmic contraction. When higher doses of each of these two agonists were applied, a mixed type of contraction with both phasic and tonic components was obtained. However, in the case of oxytocin, the contractile response disappeared in a few minutes after washing, but in the case of ARD_B it persisted for at least 30 min.

The PGE_2 derivative, Nalador, caused an increase in both amplitude and rhythmicity. All concentrations of PGE_2 provoked rhythmic contraction, and each phasic contraction was prolonged for a few seconds by a tonic component. The frequency of phasic contractions was dose-dependent, and the contractile response persisted after washing (40-60 min for high doses).

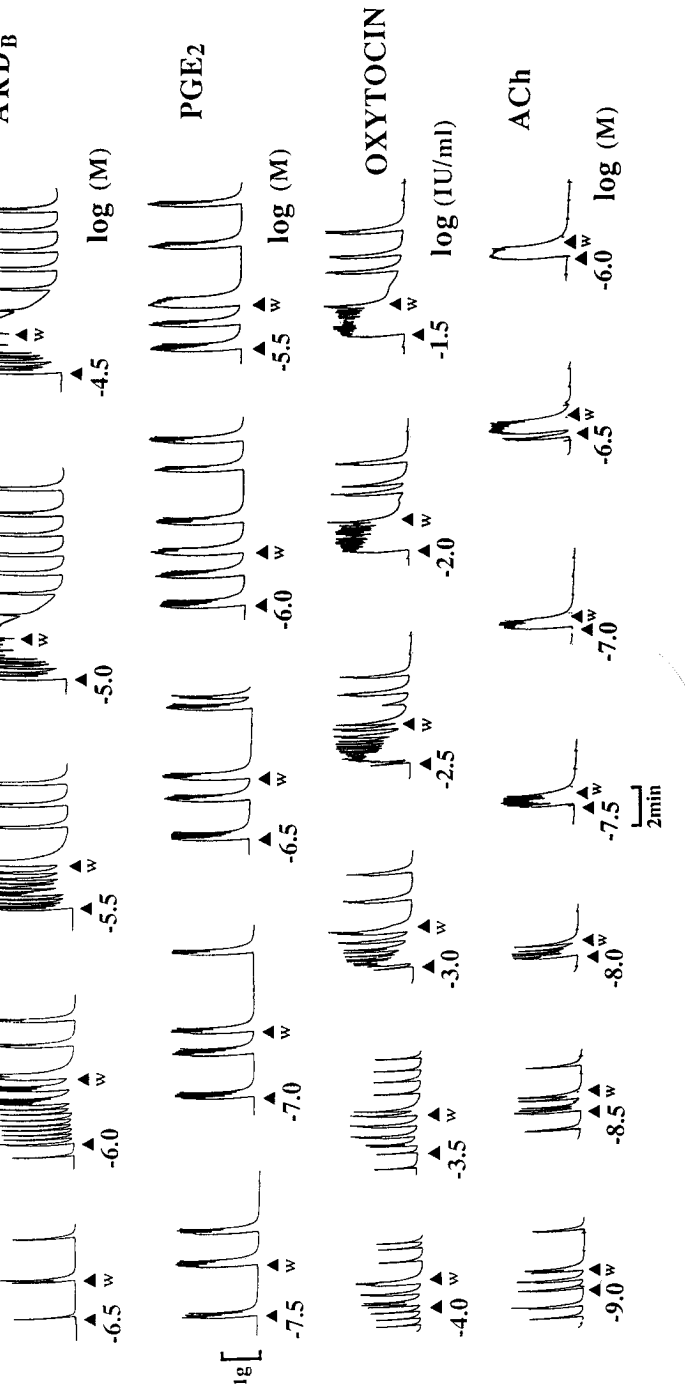


Fig. 2. Typical contractile responses to ardisiacrispin B (ARD_B), prostaglandin E_2 (PGE_2), oxytocin and acetylcholine (ACh) of uterine smooth muscle. w indicates washing.

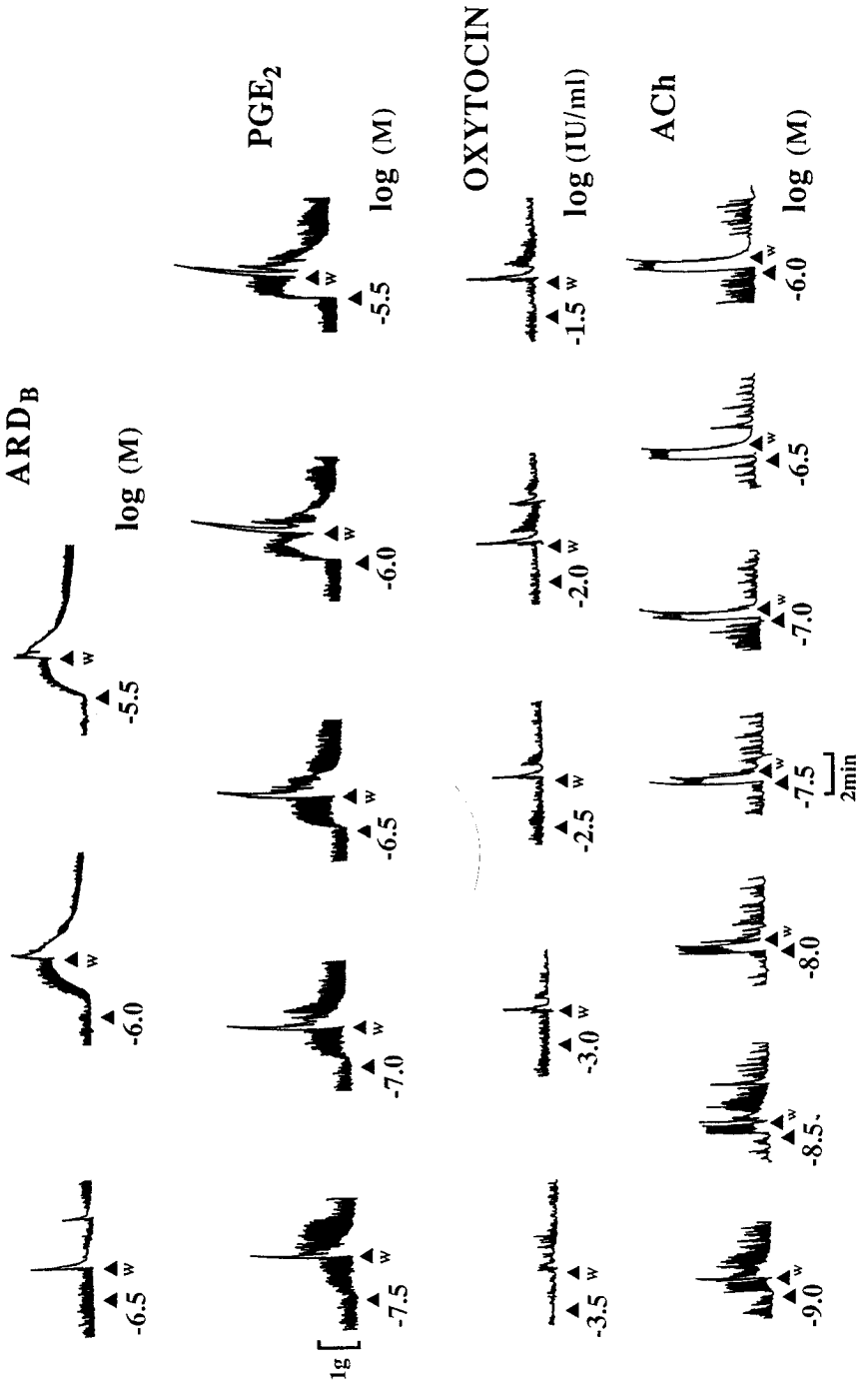


Fig. 3. Typical contractile responses to ardisiacrispin B (ARD_B), prostaglandin E_2 (PGE_2), oxytocin and acetylcholine (ACh) of small intestine. w indicates washing.

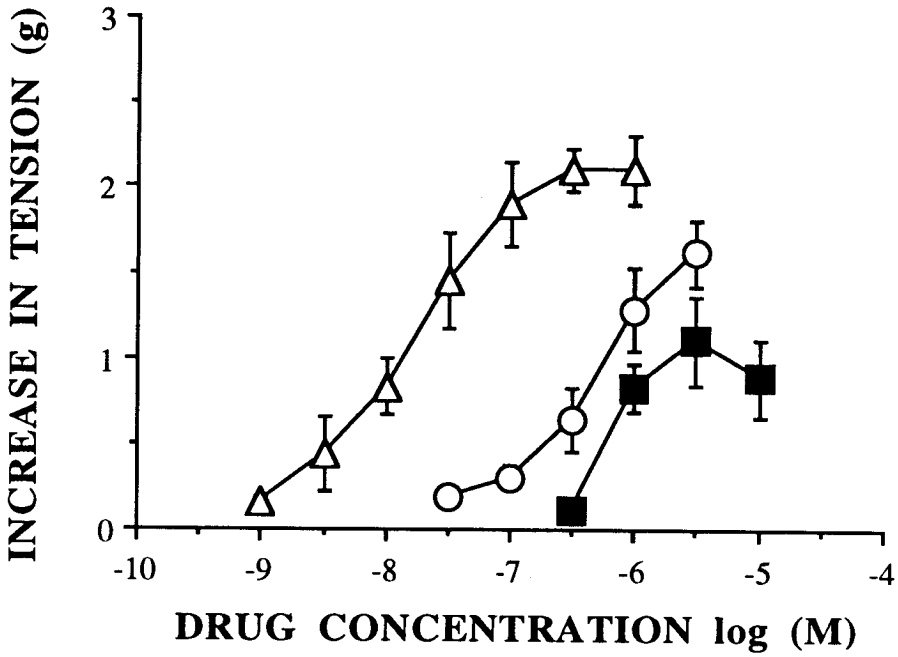


Fig. 4. Constrictor responses to ardisiacrispin B (ARD_B), prostaglandin E_2 (PGE_2) and acetylcholine (ACh) of small intestine obtained from non-pregnant rats. Each point represents the mean±s.e. mean of data from 4 experiments. (Δ) ACh; (○) PGE_2 ; and (■) ARDB.

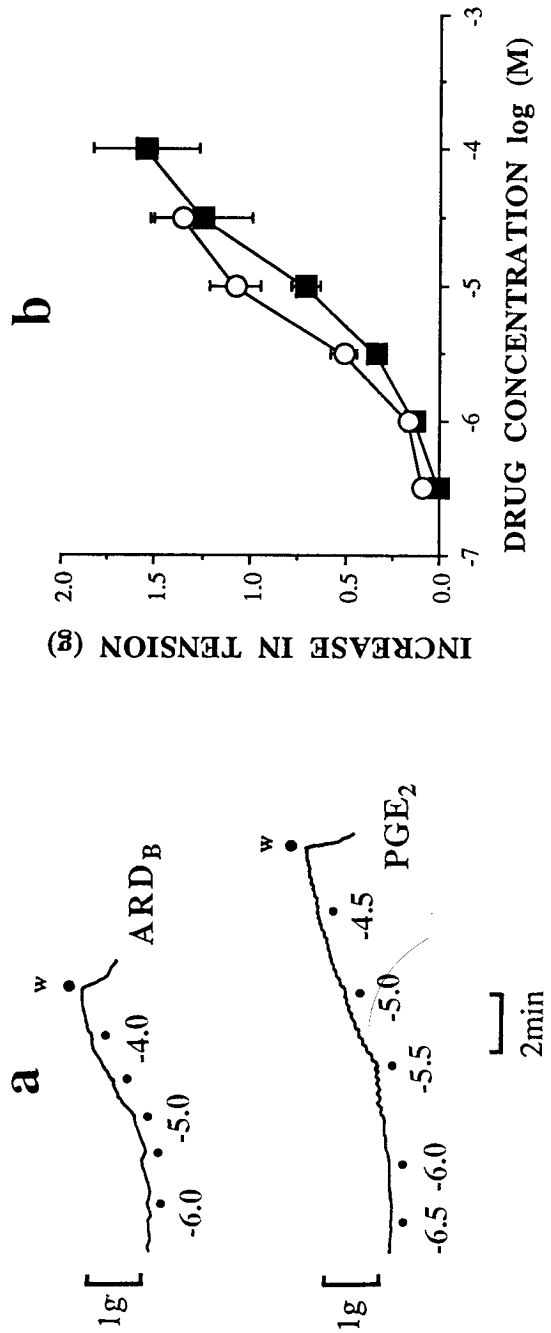


Fig.5. (a) Typical tracers of contractile responses, and (b) constrictor responses to ardisiacrispin B (ARD_B) and prostaglandin E_2 (PGE_2) of thoracic aortic rings with endothelium-intact obtained from non-pregnant rats. Each point represents the mean±s.e.mean of data from 4 experiments. (■) ARD_B and (○) PGE_2. w indicates washing

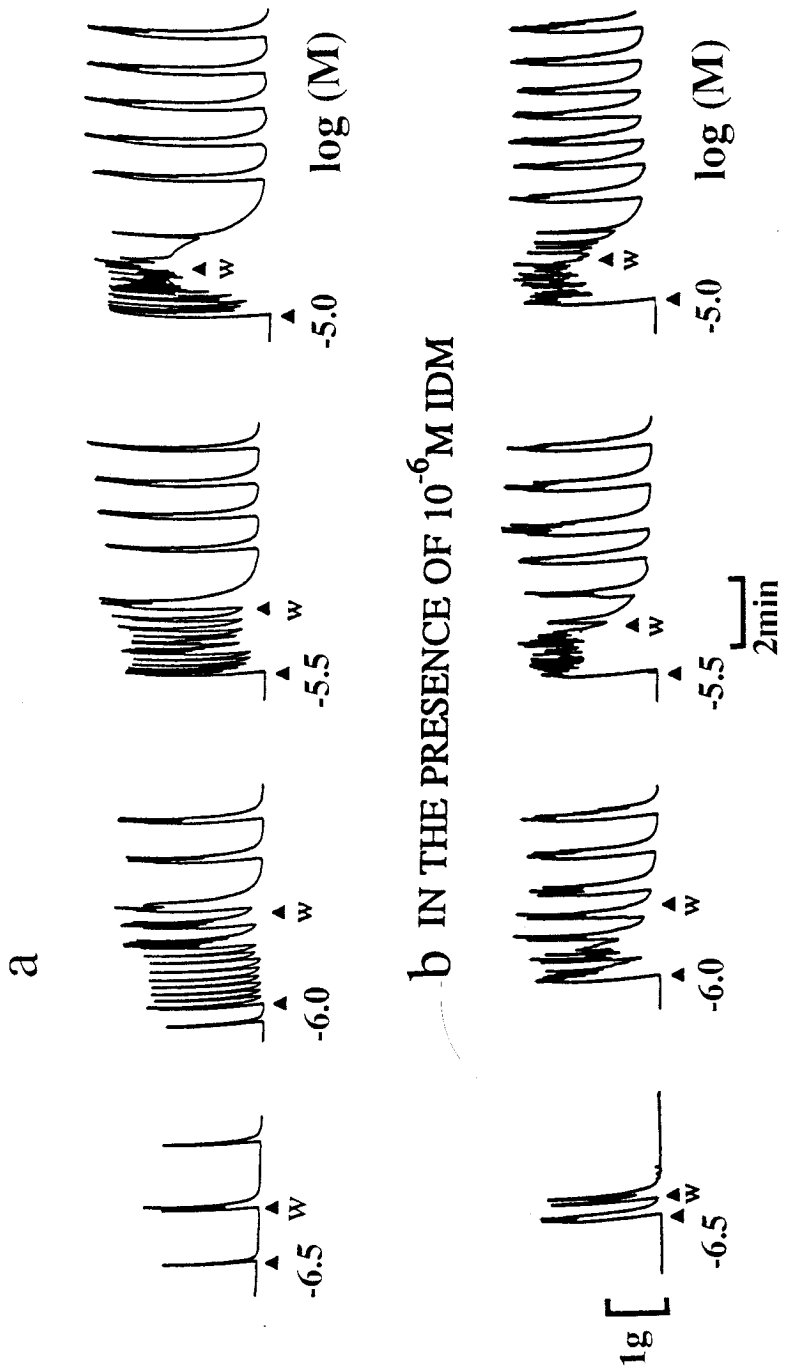


Fig. 6. Typical contractile responses to ardisiacrispin B of uterine smooth muscle in the absence (a) or presence (b) of 10^{-6} M indomethacin (IDM). w indicates washing.

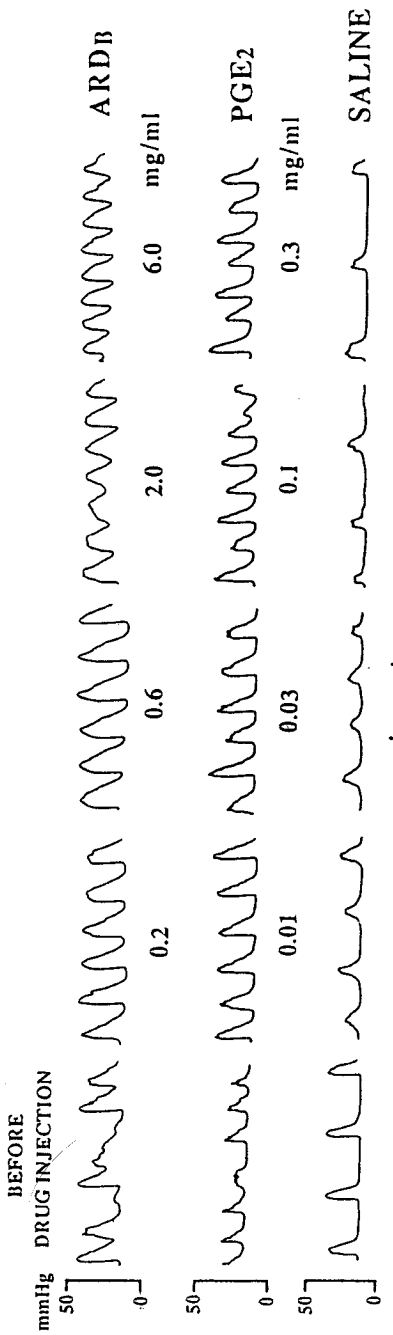


Fig. 7. Typical tracers of the effects of intra-uterine injection of ardisiacrispin B ($ARD_{B)}$, prostaglandin E_2 (PGE_2) and saline on spontaneous uterine contractility of anesthetized rats *in situ*. Each episode shows only a few minutes of the maximal activity of each concentration of the drugs. In the case of saline, 0.05 ml normal saline (0.9% NaCl, w/v) was injected at the same time as that ARD_B or PGE_2.

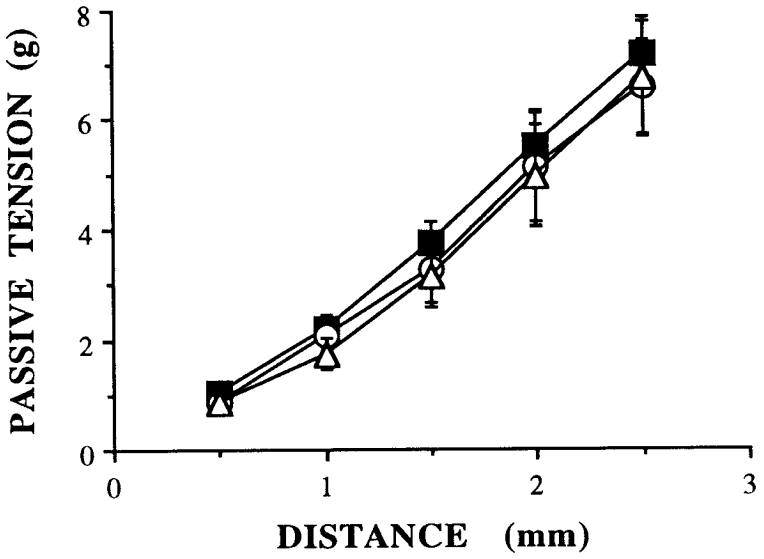


Fig. 8. Luminal expanded distances and passive tension relationship of cervical tissues *in vitro* after exposure to ardisiacrispin B (ARD_B), prostaglandin E_2 (PGE_2) or saline *in situ*. Each point represences the mean ± s.e.mean of data from 6-8 experiments. (■) ARD_B; (○) PGE_2, and (Δ) saline.

Low doses of ACh caused an increase in frequency of phasic contraction, moderate doses caused an increase in both phasic and tonic contraction, while high doses of the drug provoked a complete tetanic contraction. However, all of these effects disappeared after washing.

Fig. 3 shows typical contractile responses of small intestine to ARD_B, PGE_2, oxytocin and ACh. The effects of the ARD_B, PGE_2 and ACh are dose-dependent. Oxytocin had no effects on small intestine in any doses studied ((-3.5)-(-1.5) log (I.U/ml)). The contractile responses to ARD_B of small intestine are similar to those obtained from PGE_2. The contractile response to ACh was rapid and disappeared immediately after washing.

The DR-curve to ARD_B, PGE_2 and ACh of small intestine are shown in Fig.4. The maximal contractile response to ACh is significantly higher than those obtained from ARD_B or PGE_2 ($p<0.05$). The EC_{50} value of ACh is 0.01 (0.007-0.02, c.l.) uM. Although the maximal contractile response to PGE_2 is higher than those obtained from ARD_B, they were not statistically significant difference. The EC_{50} values for PGE_2 and ARD_B are 0.4 (0.2-0.8, c.l.) and 0.9 (0.3-2.3, c.l.) uM, respectively. The activity on small intestine of ACh is about 270 and 61fold more potent than that of PGE_2 and ARD_B, respectively.

Both ARD_B and PGE_2 caused contraction of isolated thoracic aorta with intact-endothelium in a dose-dependent manner. The typical contractile responses to thoracic aorta of both drugs are shown in Fig.5 (a) and the DR-curve are shown in Fig. 5 (b). The patterns of contraction of thoracic aortae in response to ARD_B and PGE_2 are similar, as are the DR-curves. The ED_{50} value of ARD_B is 9.5 (5.9-15.0, c.l.) uM and of PGE_2 is 4.2 (2.9-5.9, c.l.) uM, which are not statistically significantly different.

The presence of 10^{-6} M indomethacin, a cyclo-oxygenase inhibitor, did not alter the contractile response to ARD_B of rat uterine smooth muscle (Fig. 6)

Typical uterine contractility to intra-uterine administration of ARD_B, PGE_2 or saline *in situ* preparations is shown in Fig.7. In the time treatment control experiments *in situ,* in which saline was injected into the vaginal lumen in the same manner at time intervals as those of ARD_B or PGE_2, the spontaneous contractility of uterine stabilzed after 1 hr of equilibration and the intra-uterine injection of saline did not modify the spontaneous contractility.

ARD_B (0.2-6.0 mg/ml) caused increases in both rhythmicity and tonic contraction of the uterus in a dose-dependent manner. While PGE_2 (0.01-0.3 mg/ml) caused an increase in both rhythmicity and amplitude of uterine contraction, PGE_2 has no effects on blood pressure (data not shown). High dose of ARD_B (6.0 mg/ml), however, caused a lowering blood pressure (16.65 ± 7.8 mmHg, n=8) in some animals (4 out of 8).

Results of the indirect study of cervix softening are shown in Fig.8. Neither ARD_B nor PGE_2 induced any decrease in passive tension at any degree of stretching of the cervical lumen, when compared to those obtained by saline injection control.

DISCUSSION

To characterize the pharmacologic action of ARD_B, a comparison was made on the patterns of contraction and DR-curves provoked by PGE_2, oxytocin and ACh , the known smooth muscle spasmotic drugs, on the uterine, small intestine and thoracic aorta obtained from estrous rats. In the present study, the patterns of uterine contraction provoked by oxytocin, prostaglandin and acetylcholine are similar to those reported in near-term pregnant rats[9-10].

Furchgott and Zawadzki (1980)[11] have shown that acetylcholine has no effects on thoracic aortae with intact endothelium obtained from rats or rabbits, but caused vasodilatation in rings preconstricted with noradrenaline. In the present study, ARD_B caused vasoconstriction of endothelium-intact thoracic aortae of the rat. Moreover, the contractile responses of uterine smooth muscle or small intestine to ARD_B are different from those effected by ACh. Thus, an acetylcholine-like pharmacologic action of ardisiacrispin B should be ruled out.

The contractile pattern of ARD_B on rat uterine muscle is quite similar to that provoked by oxytocin (see Fig.2). However, the contractile response to oxytocin on uterine muscle was abolished immediately after washing while those caused by ARD_B persisted for a long period. Moreover, ARD_B also caused contraction of small intestine while this effect was not found when using oxytocin as an agonist. Thus, it is unlikely that ARD_B exerts its effects as an oxytocin-like activity.

Although the pattern of contractile activity of ARD_B on rat uterine smooth muscle is not similar to those provoked by PGE_2 (see Fig.2), both agonists had a long-lasting effect on the muscle after washing, which was not found in the case of oxytocin or ACh. The pattern of contraction of different kinds of smooth muscle eg. small intestine or thoracic aorta, to ARD_B and PGE_2 were similar. Beside this, the dose-response relationship to both agonists on small intestine, as well as on thoracic aortae, are similar: the two curves are parallel with no difference in the maximal contractile responses. Although the ED_{50} value of PGE_2 seems to be less than those of ARD_B, the difference was not statistically significant. These results suggest that ARD_B may exert a prostaglandin E_2-like activity.

In order to confirm that ARD_B has a PGE_2-like effect, the next experiment was designed to test the ability of the compound to exert a local effect on spontaneous contractility and cervix softening *in situ* and *in vitro*, respectively. As shown in Fig. 7 and 8, both PGE_2 and ARD_B cause an increase in spontaneous contraction of the uterus, although the patterns of the contractility are different: ARD_B caused an increase in both rhythmicity and tonus, while PGE_2 increased rhythmicity and amplitude. However, the present study failed to demonstrate that PGE_2 and ARD_B have any acute cervix softening activity. The reasons for this may be (1) the method using is not sensitive enough to detect a sophisticated change of the cervical tissues, and/or (2) the period of time that the drugs were allowed to contact the cervical tissue (2hrs) was not long enough for the cervical tissues to develop softening, which involves some specific enzymes to depolymerize and degrade the collagen of the cervix[12]. In pregnant human, it takes 12-16 hrs after having insertion of a vaginal suppository of PGE_2-gel to be able to detect any cervical dilatation[5-6]. Therefore, further study involving

cervix softening in non-pregnant rats with longer periods of drug-tissue exposure of both PGE_2 and ARD_B are neccessary.

In conclusion, ARD_B may exert a PGE_2-like effect which may act at the prostaglandin E_2-receptor but not by stimulation or enhancement of prostaglandin synthesis. The compound may be useful to develop to use as a local abortifacient drug. However, further studies on its cervix softening activity in non-pregnant rats as well as on direct abortifacient activity in pregnant animals should be undertaken.

ACKNOWLEDGEMENTS

This research was funded by the International Foundation for Science, Sweden.

The author like to thank Dr. Alan Geater, Prince of Songkla University, for contributions made in reviewing the manuscript.

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บทคัดย่อ

การศึกษาครั้งนี้มีวัตถุประสงค์ที่จะศึกษาถึงคุณลักษณะการออกฤทธิ์ในทางเภสัชวิทยาของ ardisiacrispin B (ARD_B) ทำการทดลองแบบ *in vitro* โดยใช้กล้ามเนื้อมดลูก ลำไส้เล็ก และหลอดเลือดแดงใหญ่บริเวณทรวงอกของหนูขาวใหญ่เพศเมียระยะอีสตรัส โดยดูความสัมพันธ์ระหว่างขนาดของยากับการตอบสนองของกล้ามเนื้อดังกล่าวต่อ ARD_B, prostaglandin E_2-derivative (Nalador, PGE_2), oxytocin และ acetylcholine (ACh) ศึกษาผลของ ARD_B ต่อการกระตุ้นให้มดลูกมีการสร้าง prostaglandins ซึ่งทำการทดลองโดยศึกษาผลของ ARD_B ต่อกล้ามเนื้อมดลูก ในขณะที่กล้ามเนื้อมดลูกถูกยับยั้งการสร้าง prostaglandins โดยสาร indomethacin ซึ่งเป็นสารที่ยับยั้งการทำงานของ cyclo-oxygenase นอกจากนี้ศึกษาผลแบบจำเพาะที่ของ ARD_B ที่มีต่อการบีบตัวได้เองของมดลูกในหนูขาวใหญ่แบบ *in situ* และผลต่อการทำให้ปากมดลูกมีการอ่อนตัว โดยตัดเอาส่วนของปากมดลูกมาศึกษาแบบ *in vitro*

ARD_B มีผลทำให้มีการหดตัวของมดลูก ลำไส้เล็ก และหลอดเลือดแดงใหญ่ ความแรงในการตอบสนองขึ้นกับขนาดของยา และให้ผลคล้ายกับ PGE_2. Oxytocin มีผลทำให้มดลูกหดตัวเช่นกัน แต่ไม่มีผลต่อการหดตัวของลำไส้เล็ก ACh มีผลทำให้มดลูกและลำไส้เล็กมีการหดตัว แต่ลักษณะของการหดตัวของกล้ามเนื้อทั้งสองแตกต่างไปจากการหดตัวเนื่องจาก ARD_B. Indomethacin ไม่มีผลทำให้เกิดการเปลี่ยนแปลงการตอบสนองของมดลูกต่อ ARD_B ส่วนการศึกษาในสัตว์ทดลองแบบ *in situ* โดยการฉีดยาเข้าที่โพรงมดลูกโดยตรง ทั้ง ARD_B และ PGE_2 มีผลทำให้เพิ่มการบีบตัวได้เองของมดลูก และความแรงในการตอบสนองขึ้นอยู่กับขนาดของยาที่ฉีดเช่นเดียวกับ PGE_2 ไม่มีผลต่อความดันโลหิต ยกเว้น ARD_B ที่ขนาดสูงสุด (6 มก/มล) มีผลทำให้ลดความดันโลหิตในสัตว์ทดลองบางตัว การฉีด ARD_B หรือ PGE_2 เข้าในโพรงมดลูก ไม่พบว่ามีผลทำให้ปากมดลูกอ่อนตัว เมื่อเปรียบเทียบผลกับกลุ่มที่ฉีดน้ำเกลือ ผลการทดลองเหล่านี้เป็นการชี้แนะว่า ARD_B อาจจะแสดงผลคล้ายกับ PGE_2 โดยที่ ARD_B อาจจะไปจับที่ receptor ของ PGE_2 โดยตรง ไม่ใช่เป็นผลโดยการไปกระตุ้น หรือไปเสริมให้เนื้อเยื่อมีการสร้าง PGE_2