EFFECT OF JAPANESE ANGELICA ROOT AND PEONY ROOT ON UTERINE CONTRACTION IN THE RABBIT *IN SITU*

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Intraduodenal administration of 70% MeOH extract of Japanese Angelica root (3 g/kg) and Peony root (3 g/kg) increased uterine contractile activities in anesthetized rabbits. The activities of both extracts shifted to the aqueous layer (positive at a dose of 1 g/kg) with successive fractionation of the extracts with hexane and BuOH, indicating contribution of a hydrophilic principle(s) to the effect. In some animal preparations, inhibiting effect of the extracts on uterine contraction was noted after the uterotonic effect terminated. The aqueous fraction (40 mg/kg) after being treated with hexane and BuOH also produced uterine contraction along with decrease of blood pressure through *i.v.* route, which was greatly inhibited by pretreatment of atropine, suggesting participation of cholinergic components in the effect of both extracts.

Keywords—atropine; blood pressure; extract; Japanese Angelica root; Peony root; uterine contraction

INTRODUCTION

Japanese Angelica root and Peony root are important crude drugs which have frequently been used in Chinese medicine as one member of ingredients constituting a lot of prescriptions for treatment of diseases especially in women. Concerning pharmacological studies, spasmolytic effect of ligustilide,¹⁾ a component of Japanese Angelica root, anti-nociceptive effect of polyacetylene compounds like falcarinol,²⁾ a component of the same crude drug, a large number of pharmacological effects of paeoniflorin,³⁾ a component of Peony root and a lot of studies on extract of both crude drugs⁴⁾ have been reported. However, influences of extract of both crude drugs administered in the digestive tract on the female sex organ have not been known in detail. In the present paper, with reference to the clinical practice, extract of both crude drugs on uterine contraction *in situ* was studied in the rabbit through the duodenal application of it.

MATERIAL AND METHODS

Materials—Commercial Japanese Angelica root (JPX) and Peony root (JPX) were used, mother plants of which were cultivated in Toyama and Nara prefectures, respectively. Crude extract and fractionated extract were prepared according to a flow diagram given in Chart 1.

In the case of intraduodenal administration, 70% MeOH extract, the first aqueous extract (not treated with CHCl₃ and BuOH in the case of Japanese Angelica root and Peony root, respectively) and the second aqueous extract (treated with BuOH and separated from BuOH layer) of both crude drugs were dissolved in distilled water and BuOH extract of both crude drugs and hexane extract of Peony root were suspended in a 4% acacia solution to give a test solution. Hexane extract of Japanese Angelica root was used *per se* because of its fluid state. In the case of *i.v.* administration, the second aqueous

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extract of both crude drugs was centrifuged (3000 rpm, 20 min) to make a test solution.

Animals — Female rabbits weighing 2.3–3.2 kg were *s.c.* injected with diethylstilbestrol (0.2 mg/kg/d $\times$ 4). They were fasted overnight with free access to water on the final day of the hor-

mon treatment for the experiment on the following day.

Pharmacological Methods — 1) Intraduodenal Administration: Rabbits were anesthetized with urethane (0.85 g/kg, *i.v.* plus 0.85 g/kg, *s.c.*). Tracheal intubation and cannulation of the right

CHART 1. Flow Diagram of the Fractionation of Crude Drugs

a) expressed as the first aqueous extract in the text.

b) expressed as the second aqueous extract in the text.

femoral vein for drug injection were made. Under laparotomy, a rubber tube for injection of a test solution was distally introduced in the duodenum 5 cm away from the fundus and a small-sized probe of a strain gauge (Tokai I-rika) was fixed on muscle tissues of a uterine horn. Then, the opened abdomen was closed by suture. Uterine contraction was recorded on an ink-writing recorder *via* the strain gauge. Approximately 30 min after completion of the surgical operation, oxytocin, 100 mU/kg, was *i.v.* administered to check sensitivity of the organ. Then, at 90-min intervals, saline solution which served as control, a test solution and 10–20 mg/kg of papaverine which served as a referential drug were successively administered. Responses of the organ to papaverine was observed for 60 min. Test solutions were given at volumes of 15 ml/animal for 2 min.

2) *i.v.* Administration: The animal preparation was provided by the same procedure as above, except with no intraduodenal cannulation and with cannulation of the left common carotid artery for recording of blood pressure *via* a pressure transducer (MPU-0.5, Nihon Kohden). Ap-

proximately 2 h after completion of the surgical operation, oxytocin, 100 mU/kg, was *i.v.* injected. Then, at 30-min intervals, 40 mg/kg of an extract and 10^{-7} mol/kg of acetylcholine were successively administered. One hour later, 2 mg/kg of atropine was administered and successive injection of the same dose of the extract and acetylcholine as above was made at 5-min intervals.

Evaluation of Uterine Contraction — 1) Intraduodenal Administration: One experimental period ranging from 10 min before, to 90 min

FIG. 1. *Effect of Japanese Angelica Root on Uterine Contractile Activities in the Rabbit in Situ*
At an arrow, 3 g/kg of 70% MeOH Extract was intraduodenally administered.

TABLE I. *Effect of 70% MeOH Extract of Japanese Angelica Root on Uterine Contractile Activities*

Time (min) ^{a)}	Control (N=5)	Extract (3 g/kg, N=5)	Papaverine (10 mg/kg, N=5)
5	85.4 ± 3.3 ^{b)}	95.2 ± 14.9 ^{b)}	77.8 ± 16.3 ^{b)}
10	87.4 ± 11.0	96.4 ± 25.7	43.8 ± 17.9
15	105.4 ± 12.5	168.4 ± 56.3	37.6 ± 16.2 ^{c)}
20	115.8 ± 16.2	152.8 ± 55.0	40.2 ± 17.3 ^{c)}
25	106.0 ± 15.6	170.2 ± 45.6	61.6 ± 18.0
30	110.6 ± 15.0	155.2 ± 33.9	55.4 ± 11.1 ^{c)}
40	120.8 ± 22.8	139.4 ± 32.7	93.2 ± 11.3
50	96.2 ± 20.7	143.4 ± 22.0	88.4 ± 10.3
60	79.8 ± 21.4	101.2 ± 28.6	98.4 ± 18.9
70	89.8 ± 26.5	93.0 ± 12.2	
80	77.8 ± 9.6	119.2 ± 24.5	
90	65.6 ± 21.1	124.6 ± 34.6	

a) Drugs were administered at time 0.

b) % to the value at time 0 (1.80 ± 0.86 g, 1.39 ± 0.55 g, 1.83 ± 0.63 g, respectively).

c) $p < 0.05$.

after, drug administration is divided into 5-min intervals. Summation of each contractile peak measured in tension (g) during each 5-min period was regarded as contractile activities for the period. Value of the activities was expressed in terms of percentages to the averaged value before the drug administration.

2) *i.v.* Administration: The maximal contractile peak expressed in tension (g) during 2 min before, and 2 min after, drug administration was regarded as contractile activities.

Statistics — Student's *t* test (for intraduodenal administration) and paired *t* test (for *i.v.* administration) were used. All results were expressed as mean $\pm$ s.e.

Drugs — Drugs used were oxytocin injection (Teikoku Zoki), atropine sulfate (Wako Junyaku Kogyo), acetylcholine chloride (Daiichi Kagaku Yakuhin), diethylstilbestrol (Tokyo Kasei Kogyo) and papaverine hydrochloride (Iwaki). Doses of the base below were expressed in terms of the salt.

RESULTS

1) Intraduodenal Administration

Typical recordings of contractile activities of 3 g/kg of 70% MeOH extract of Japanese Angelica root are given in Fig. 1 and all results summarized in Table I. Uterine contractions increased 15 min after administration of the extract, reaching a maximal level of approximately 70% of the original level and returned to the original level in 60 min. Thereafter, decrease of contractile activities was shown depending upon the individual of the animal. In contrast, papaverine inhibited contractions by approximately 60% in 10–30 min after the administration. Hexane fraction, 0.1–1 g/kg and BuOH fraction, 0.3 g/kg gave rise to no marked influence on the uterine activities. On the contrary, as shown in Fig. 2 and Table II, 1 g/kg of the first aqueous fraction increased the contractions in 15–40 min after the administration, with (Fig. 2, lower) or without (Fig. 2, upper) succeeding decrease of them. The same dose of the second aqueous fraction, however, did not show such

marked effects as shown in the first aqueous fraction in the present experiment ($n=4$, data not given).

Typical recordings of contractile activities of 3 g/kg of 70% MeOH extract of Peony root are illustrated in two patterns, *i.e.*, increase only (Fig. 3, upper) and increase followed by decrease (Fig. 3, lower). All results are summarized in Table III. The contractions increased by 50–60% in 10–15 min after the administration. Then, the activities was reduced below the original level. Inhibitory effect of papaverine was shown although the potency was weaker compared with that at the experiment on Japanese Angelica root. Effect of 0.05–0.5 g/kg of hexane fraction and 0.5 g/kg of BuOH fraction was not observed. In contrast, as shown in Table IV, 1 g/kg of the second aqueous extract increased the contractions 10 min after the administration, sustaining the activities more than 30 min thereafter.

2) *i.v.* Administration

Typical recordings of the activities exerted with 40 mg/kg of the second aqueous extract of Japanese Angelica root and 40 mg/kg of the second aqueous extract of Peony root are shown in Fig. 4, upper and lower, respectively, and all results summarized in Table V. Both extracts as well as acetylcholine contracted the uterine

FIG. 2. Effect of Japanese Angelica Root on Uterine Contractile Activities in the Rabbit in Situ
At an arrow, 1 g/kg of the first aqueous extract was intraduodenally administered.

TABLE II. *Effect of the First Aqueous Extract of Japanese Angelica Root on Uterine Contractile Activities*

Time (min) ^{a)}	Control (N=5)	Extract (1 g/kg, N=5)	Papaverine (20 mg/kg, N=2)
5	76.4 ± 11.8 ^{b)}	134.4 ± 24.5 ^{b)}	87.5 ^{b)}
10	81.6 ± 5.0	101.2 ± 11.0	53.0
15	86.8 ± 6.9	145.2 ± 13.6 ^{c)}	26.0
20	88.0 ± 11.1	151.0 ± 16.9 ^{d)}	28.5
25	82.6 ± 9.6	150.6 ± 27.6 ^{d)}	42.5
30	99.0 ± 5.0	158.2 ± 13.8 ^{c)}	41.0
40	85.6 ± 8.4	161.6 ± 21.3 ^{d)}	56.5
50	80.0 ± 6.9	146.0 ± 42.1	115.5
60	77.8 ± 12.8	136.2 ± 57.3	114.5
70	59.6 ± 9.3	134.8 ± 54.6	
80	75.6 ± 7.0	150.4 ± 53.3	
90	58.2 ± 5.5	152.0 ± 49.1	

a) Drugs were administered at time 0.

b) % to the value at time 0 (1.18 ± 0.22 g, 0.76 ± 0.15 g, 0.79 ± 0.07 g, respectively).

c) $p < 0.01$.

d) $p < 0.05$.

muscle and decreased blood pressure. Pre-administration of 2 mg/kg of atropine greatly inhibited these effects of both extracts and the original effects of both extracts again appeared along with the reappearance of those of acetylcholine. Antagonistic effects of 2 mg/kg of atro-

pine against uterotonic as well as hypotensive activities of the extract of Peony root were a little weaker compared with those of the extract of Japanese Angelica root.

DISCUSSION

Intraduodenal administration of 70% MeOH extract of Japanese Angelica root and Peony root influenced the uterine activities in anesthetized rabbits. Increase of uterine contractions was produced by both extracts and, especially in the latter extract, inhibitory effect on them was noted in the later part of the observation period. With successive fractionation of both extracts with hexane and BuOH, the contractile activities of both extracts shifted to the aqueous layer, no practical ones being left in any layer of organic solvents. Inhibitory effect on the uterine contractions was also observed in the aqueous fraction in some animal preparations. Therefore, it is considered that substance(s) responsible to these effects, excitatory and inhibitory, may be hydrophilic.

Considering the component complexity of

FIG. 3. *Effect of Peony Root on Uterine Contractile Activities in the Rabbit in Situ*

At an arrow, 3 g/kg of 70% MeOH Extract was intraduodenally administered.

TABLE III. *Effect of 70% MeOH Extract of Peony Root on Uterine Contractile Activities*

Time (min) ^{a)}	Control (N=5)	Extract (3 g/kg, N=4)	Papaverine (10 mg/kg, N=4)
5	104.4±16.0 ^{b)}	89.8±14.9 ^{b)}	108.0±17.9 ^{b)}
10	92.2± 7.7	156.0±24.6 ^{c)}	106.3±15.1
15	94.2± 6.2	150.2±23.8	59.0±24.1
20	81.0± 8.7	112.2±17.6	92.0±31.6
25	94.0±10.3	87.0±19.2	99.3±35.6
30	88.6± 4.8	64.2±28.1	83.8±21.6
40	86.0± 6.8	55.4±24.9	101.0±17.4
50	78.2± 8.7	50.2±21.0	99.5±15.9
60	67.4± 7.9	47.2±15.4	102.8±14.2
70		43.5±15.2	
80		54.8±19.1	
90		73.8±34.8	

a) Drugs were administered at time 0.

b) % to the value at time 0 (2.08 ± 0.44 g, 1.78 ± 0.47 g, 1.63 ± 0.45 g, respectively).

c) $p < 0.05$.

TABLE IV. *Effect of the Second Aqueous Extract of Peony Root on Uterine Contractile Activities*

Time (min) ^{a)}	Control (N=5)	Extract (1 g/kg, N=5)	Papaverine (20 mg/kg, N=5)
5	93.0± 9.0 ^{b)}	119.0±13.9 ^{b)}	128.2±48.4 ^{b)}
10	89.2± 4.1	145.8±21.0 ^{c)}	56.8±26.9
15	76.4±15.9	152.0±19.8 ^{c)}	56.8±21.0
20	66.6± 9.2	182.4±50.8	57.0±19.0
25	93.4± 8.6	178.6±57.3	70.8±22.9
30	86.6± 7.2	156.0±27.0 ^{c)}	81.0±21.2
40	87.4±16.5	154.4±26.6	72.0±12.9
50	68.4±11.6	160.6±29.6 ^{c)}	80.6± 9.9
60	75.8±11.5	226.0±66.7	101.0±27.5
70	73.4± 9.6	172.0±55.0	
80	76.2±10.5	203.4±64.9	
90	66.0±12.1	186.4±68.5	

a) Drugs were administered at time 0.

b) % to the value at time 0 (0.78 ± 0.12 g, 0.81 ± 0.18 g, 0.82 ± 0.09 g, respectively).

c) $p < 0.05$.

the extracts and the complex processes in the body until appearance of pharmacological effects of the extracts after absorption of them in the digestive tract, experiment on the second aqueous fraction of both crude drugs through *i.v.* route was adopted in order to obtain further

knowledge on property of the uterotonic activities.

As expected, *i.v.* administration of the samples contracted the uterine muscle. Antagonistic experiments using atropine suggest that cholinergic component(s) participates in this event.

FIG. 4. Effect of Japanese Angelica Root (A-ext, Upper) and Peony Root (P-ext, Lower) on Uterine Contractile Activities in the Rabbit in Situ

The second aqueous extract, 40 mg/kg, was i.v. administered.

Ach: acetylcholine, Atr: atropine.

TABLE V. Effect of Aqueous Extract of Japanese Angelica Root and Peony Root on Uterine Tone

		Uterine contraction (g)		Blood pressure (mmHg)	
		Before atropine	After atropine	Before atropine	After atropine
Japanese Angelica root	Basal tone	0.85 ± 0.22	0.12 ± 0.05	96 ± 9	86 ± 9
	Extract	$1.87 \pm 0.25^a)$	0.16 ± 0.09	$78 \pm 10^a)$	$80 \pm 10^c)$
	Δ	1.01 ± 0.13	$0.09 \pm 0.09^b)$	-19 ± 3	$-7 \pm 2^d)$
	Acetylcholine	0.61 ± 0.24	0.11 ± 0.07	98 ± 7	84 ± 10
Peony root	Drug	1.44 ± 0.51	0.20 ± 0.12	$26 \pm 1^a)$	76 ± 10
		0.83 ± 0.31	0.09 ± 0.06	-71 ± 7	$-9 \pm 8^e)$
	Basal tone	0.56 ± 0.16	0.07 ± 0.08	101 ± 2	86 ± 5
	Extract	$1.47 \pm 0.11^f)$	0.32 ± 0.14	$80 \pm 5^a)$	$75 \pm 5^c)$
Acetylcholine	Δ	0.91 ± 0.22	0.25 ± 0.10	-21 ± 3	$-11 \pm 3^d)$
	Basal tone	0.36 ± 0.17	0.00 ± 0.00	95 ± 3	86 ± 5
	Drug	$1.32 \pm 0.26^a)$	0.15 ± 0.07	$53 \pm 7^f)$	81 ± 7
	Δ	0.97 ± 0.13	$0.15 \pm 0.07^d)$	-42 ± 8	$-5 \pm 3^e)$

Dose (i.v.); Japanese Angelica root and Peony root; 40 mg/kg.

a) $p < 0.01$ against basal tone, b) $p < 0.01$ against the value before atropine, c) $p < 0.05$ against basal tone after atropine, d) $p < 0.05$ against the value before atropine, e) $p < 0.01$ against the value before atropine, f) $p < 0.05$ against basal tone.

Although there is much possibility on an idea that the uterotonic activity induced by intraduodenal administration of both extracts and that induced by *i.v.* administration of them originate from the same substance(s), their identity remains to be clarified.

Finally, it seems significant for the pharmacological study on the therapeutic effect of both crude drugs that a dose of 1 g/kg of the material as crude extractive state showed pharmacological effect through administration in the digestive tract.

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