The Journal of Applied Pharmacology, **11**, 183-189(2003)

General Pharmacology of IH-901

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(Received July 9, 2003; Accepted September 18, 2003)

Abstract – General pharmacological properties of IH-901, a new pharmacological composition as an intestinal metabolite formed from ginseng protopanaxadiol saponins, were investigated in experimental animals administered orally and *in vitro* test system. IH-901 had no effects on general behavior, pentobarbital sleeping time, spontaneous motor activity, motor coordination of mice, normal body temperature, chemoshock produced by pentylenetetrazole and writhing syndromes induced by 0.8% acetic acid at the dose of 25 and 250 mg/kg. Gastric secretion of rats and intestinal motility in mice were not also influenced by the administration of IH-901 at doses of 25 and 250 mg/kg. IH-901 (25 and 250 mg/kg) did not change the mean arterial blood pressure and heart rate in conscious rats. IH-901 had no effect on the respiratory rate at the same doses when it was given to anesthetized rats. In *in vitro* experiments, IH-901 at the concentration of 25 μg/L did not show any direct effect and inhibitory or augmentative action on the histamine- or acetylcholine-induced contractions in the isolated ileum of guinea-pig. Based on these results, it was concluded that IH-901 did not induce any adverse effects in experimental animals.

Keywords □ IH-901, General Pharmacology

Ginseng saponins (ginsenosides) are regarded as the principal component responsible for the pharmacological activities of ginseng. Ginsenosides are composed of glucosides containing an aglycon with dammarane-type triterpene skeleton (protopanaxadiol, protopanaxatriol). Orally administered ginsenosides are detected not in as their intact form but in a metabolite form by intestinal bacteria, such as *Prevotella oris*, in blood (Hasegawa *et al.*, 1994 and 1997a; Sung *et al.*, 1995). It has been reported that protopanaxadiol-type ginsenosides such as Rb1, Rb2 and Rc are metabolized by intestinal bacteria after oral administration to their derivative 20-O-b-D-glucopyranosyl-20(s)-protopanaxadiol, which is refered to M1, compound K or IH-901. Recently, it was also found that the intestinal bacterial metabolites of ginsenosides were absorbed from the intestine and excreted into urine. So, they are most likely to be the primary active component of ginseng saponins (Sung *et al.*, 1995; Hasegawa *et al.*, 1997a).

IH-901 is a major intestinal metabolite form of ginseng protopanaxadiol saponins and has been shown to possess some pharmacological activities such as the inhibition of glucose uptake by tumor cells (Hasegawa *et al.*, 1994) and the reversal of multidrug-resistance in bacteria and tumor cells (Hasegawa *et al.*, 1997b). Also, IH-901 exhibited antitumor activities *in vivo* and *in vitro*. IH-901 inhibited the proliferation of the cancer cells such as human myeloid leukemia (HL-60), pulmonary adenocarcinoma (PC-14), gastric adenocarcinoma (MKN-45), hepatoma (HepG2), and CDDP-resistant PC/DDP cell lines (Hasegawa *et al.*, 1997b) in *in vitro*. In case of *in vivo* study, antimetastatic activity against Lewis lung carcinoma and anticarcnogenic effect against 1,2-dimethylhydrazine (DMH) in Ginseng-hydrolyzing bacteria colonized (GBC) mouse of IH-901 were confirmed (Hasegawa *et al.*, 1997b; Hasegawa and Benno, 2000). Moreover, it was found that IH-901 has antiplatelet aggregation, antiangiogenic, antigenotoxic and antiinvasive activities (Hasegawa *et al.*, 1997b; Lee *et al.*, 1998; Suda *et al.*, 2000).

In the present study, as a part of preclinical evaluation of IH-901, the general pharmacological effects of IH-901 on general behavior, central nervous system, digestive system, smooth muscles, and cardiovascular and respiratory systems were investigated.

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MATERIALS AND METHODS

Animals

Male ICR mice (20-30 g), male Sprague-Dawley (SD) rats (250-300 g), and male Hartley guinea pigs (300-350 g) were purchased from Sam:TacN(SD)BR (Osan, Keonggi, Korea). Animals were housed in an acrylfiber cage in a controlled room (temperature of 22 ± 2°C, relative humidity of 50 ± 5%), were maintained in a light-controlled room (light: 07:00-19:00, dark: 19:00-07:00) and were given solid diet and tap water *ad libitum*. Animals were grouped randomly into the groups of 3-4.

Test substance and dose selection

IH-901 was supplied by Central Research Institute, Il-Hwa Co., Ltd. (Guri-Shi, Kyonggi-Do, Korea). IH-901 showed the maximum anticancer effect, when the plasma concentration reached to 10-20 μg/ml in mice. When 50 μg of IH-901 was administered orally to mice (20 g), the plasma concentration reached to 20 μg/ml after the half-life of IH-901. Thus, the doses of 25 and 250 mg/kg, 10 and 100 folds of the effective dose in mice, were slected. The dosing solution was prepared by suspending IH-901 with 10% tween 80 solution. The 10% tween 80 solution was used as the vehicle control.

Drugs

Pentylenetetrazole, aminopyrine, chlorpromazine, hydralazine, acetylcholine and histamine dihydrochloride were purchased from Sigma (St. Louis, MO, USA). Pentobarbital sodium was obtained from Hanlim Pharm. Ind. Co. (Seoul, Korea) and phenobarbital, from Jeil Pharm. Co (Daegu, Korea). All other chemicals used were of the reagent grade.

Effects on general behavior

The methods used were based on the procedures described by Irwin (1968). Mice were administered orally with IH-901 (25 and 250 mg/kg), and the general behaviors were observed at 0.5, 1, 2 and 4 hrs after the drug administration.

Effect on central nervous system

Spontaneous locomotor activity

Each mouse was placed in an activity cage with an automatic recording device (AM1051, Benwick Electronics, Benwick, UK), and activity for 5 min were recorded at 0.5, 1, 2 and 4 hr after the drug administration. Data were shown as a percentage of the value measured before the drug administration. Chlorpromazine (3 mg/kg) was used as positive control.

Pentobarbital-induced sleeping time

One hr after the drug administration, pentobarbital (32 mg/kg) was injected intraperitoneally into the mice. The time of onset of sleep and the duration of sleeping time of each mouse were recorded. Chlorpromazine (3 mg/kg) was used as positive control.

Rotarod test

Mice were orally administered with IH-901 or vehicle and were subjected to the rotarod tests at 0.5, 1, 2 and 4 hr after the drug administration. The mice were placed on a 3 cm diameter rod (Daejong Ins. Co., Seoul, Korea) rotating at 10 rpm, and the rotarod deficit was obtained by counting the number of animals fallen from the rotating rod within 2 min, as described previously (Dunham *et al.*, 1957). Chlorpromazine (3 mg/kg) was used as positive control.

Pentylenetetrazol (PTZ)-induced convulsion

PTZ (110 mg/kg) was injected subcutaneously 1 hr after the drug administration. The induction time of the first generalized clonic seizure with loss of righting reflexes was measured. The incidence of convulsion and mortality were also determined. Phenobarbital (100 mg/kg) was used as positive control.

Analgesic activity

Each mouse was injected with 0.8 % acetic acid (0.1 ml/10 g, i.p.) 1 hr after the administration of IH-901 or vehicle, and was placed immediately in an observation cage. Ten minutes after the injection of acetic acid, the numbers of writhing episodes during the subsequent 10-min period were counted (Collier *et al.*, 1968). Aminopyrine (50 mg/kg, s.c.) was used as positive control.

Body temperature

Body temperature was measured rectally using an electrothermometer (Thermalert TH-5, Physitemp, USA). IH-901 or vehicle was administered orally to male mice (22-25 g) and rectal temperatures were measured at 0.5, 1, 2 and 4 hr after the drug administration. Chlorpromazine (3 mg/kg) was used as positive control.

Effect on gastrointestinal system

Intestinal propulsion

The mice fasted overnight were administered orally with IH-901 or vehicle. Fifty minutes after the administration of test drug, each mouse received orally 0.2 ml of 5% w/v suspension of

charcoal in 0.5% carboxymethylcellulse sodium solution. Twenty minutes after the administration of charcoal meal, the mice were sacrificed and the distance traversed by the charcoal meal along the small intestine from the pyloric sphincter was measured (Takemori *et al.*, 1969). This distance was calculated as a percentage of the total length of the gut.

Gastric acid secretion

The rats were fasted for 24 hr. Under ether anesthesia and with rats in the supine position, their abdomen was opened along the midline (Shay *et al.*, 1945). The pylorus was ligated and then IH-901 was intraduodenally given. At five hours after closing the abdomen, the rats were sacrificed and stomach was removed. The volume, pH, and free HCl and total acid concentrations of gastric juice were measured.

Effect on cardiovascular system

Mean blood pressure and heart rate in conscious rats

Male SD rats (250-350 g) were anesthetized with pentobarbital sodium (50 mg/kg, i.p.), and the polyethylene (PE-10) catheter filled with heparinized saline solution (100 IU/ml) was inserted into the carotid artery for recording arterial blood pressure and heart rate. The animals were allowed 1 day to recover and stabilize in individual cages. On the day of experiment, rats were kept moving free in individual cages in a quiet room, and the arterial catheter was connected to a pressure transducer (World Precision Instruments, Inc. (WPI) CDXIII, FL, USA) coupled to an amplifier (WPI, BPI, FL, USA) and chart recorder (Lectromed, MultiTrace 2, UK) for measuring blood pressure and to pulse ratemeter (Hugo Sachs PFM2, Germany) for heart rate. Arterial blood pressure and heart rate were monitored and recorded at the time of 0.5, 1, 1.5, 2 and 3 hr after single oral administration of IH-901. Hydralazine (50 mg/kg) was administered orally as positive control.

Effect on respiratory rate

IH-901 was given orally to rats after the intraperitoneal administration of pentobarbital sodium (40 mg/kg), just prior to the induction of anesthesia. Upon the induction of anesthesia, the respiratory rate was measured immediately. The measured number was regarded as the value prior to the administration. Respiration belt (Hugo Sachs, Germany) including respiratory sensor was attached to the abdomen of anesthetized rats, and the respiration was recorded in a chart recorder (IITC Life Science, CA, USA) through amplifier (Hugo Sachs, 2-channel bridge, Germany). Respiratory rate was monitored and recorded for 3 hrs. The number of respiration for 1 min was measured at the time of 0.5, 1, 1.5, 2 and 3 hr after the single oral administration of IH-901.

Effect on smooth muscle

Agonist-induced contractions in isolated guinea pig ileum

Segments of mesenteric plexus-longitudinal muscle, about 2-2.5 cm long obtained from male Hartley guinea pig (350-450 g) ileum, was used as previously described (Rang, 1964). Isolated segments were mounted vertically in organ baths containing 10 ml of Krebs-Henseleit bicarbonate solution (mmol/L: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1, NaH_2PO_4 0.4, $NaHCO_3$ 11.9, D-glucose 5.6) at 37°C, and aerated with 95% O_2/5% CO_2. The segments were equilibrated for about 60 minutes before experiment. The tension of the preparation was isotonically recorded with a transducer (Hugo Sachs, B40, Germany) coupled to an amplifier (Hugo Sachs, 2-channel bridge, Germany) and displayed on a chart recorder (IIRC Life Science, CA, USA). To determine the direct contractile or dilatory effect of IH-901 on smooth muscle, the segments were exposed to the drug (25 μg/ml) for 5 min, and then agonist (acetylcholine or histamine) was added into the organ bath cumulatively to examine the effect of the test drug on the contraction induced by each agonist.

Statistics

Data were expressed as the mean±SEM. The statistical significances between groups were assessed by unpaired student's *t* test or Chi-square test. Differences at p values of less than 0.05 were considered to be statistically significant.

RESULTS

Effect on general behavior

Oral administration of IH-901 at doses of 25 and 250 mg/kg showed no observable changes in behavioral, neurological and autonomic profiles in mice during 4 hrs periods (Table I).

Effect on the central nervous system

Effect on spontaneous motor activity

Spontaneous motor activity of mice was gradually reduced after oral administration of IH-901 and vehicle. IH-901 (25 and 250 mg/kg) did not affect spontaneous motor activity of mice, when compared to the vehicle-treated control group (Table II).

Effect on pentobarbital-induced sleeping time

Orally administered IH-901 (25 and 250 mg/kg) did not show any significant change of the pentobarbital-induced sleeping

Table I. Effect of IH-901 on general behavior in mice

	0.5			1			2			4 (hr)		
	A	B	C	A	B	C	A	B	C	A	B	C
Locomotor activity	0	0	0	0	0	0	0	0	0	0	0	0
Writhing response	0	0	0	0	0	0	0	0	0	0	0	0
Fighting	0	0	0	0	0	0	0	0	0	0	0	0
Convulsion	0	0	0	0	0	0	0	0	0	0	0	0
Tremor	0	0	0	0	0	0	0	0	0	0	0	0
Exophtalmos	0	0	0	0	0	0	0	0	0	0	0	0
Ptosis	0	0	0	0	0	0	0	0	0	0	0	0
Piloerection	0	0	0	0	0	0	0	0	0	0	0	0
Tail elevation	0	0	0	0	0	0	0	0	0	0	0	0
Traction	0	0	0	0	0	0	0	0	0	0	0	0
Motor incoordination	0	0	0	0	0	0	0	0	0	0	0	0
Muscle Tone	0	0	0	0	0	0	0	0	0	0	0	0
Catalepsy	0	0	0	0	0	0	0	0	0	0	0	0
Righting reflex	0	0	0	0	0	0	0	0	0	0	0	0
Pain response	0	0	0	0	0	0	0	0	0	0	0	0
Pinna reflex	0	0	0	0	0	0	0	0	0	0	0	0
Skin color	0	0	0	0	0	0	0	0	0	0	0	0
Respiration	0	0	0	0	0	0	0	0	0	0	0	0
Lacrimation	0	0	0	0	0	0	0	0	0	0	0	0
Salivation	0	0	0	0	0	0	0	0	0	0	0	0
Diarrhea	0	0	0	0	0	0	0	0	0	0	0	0
Vocalization	0	0	0	0	0	0	0	0	0	0	0	0
Death	0	0	0	0	0	0	0	0	0	0	0	0

Each value represents the number of abnormalities on general behavior (n=5).
A: Vehicle, B: IH-901 25 mg/kg, C: IH-901 250 mg/kg

Table II. Effect of IH-901 on spontaneous motor activity in mice

Drug	Dose (mg/kg, p.o)	No. of animal	Activity (% of Prevalue)			
			0.5 hr	1hr	2hr	4hr
Vehicle	-	12	60.6±12.3	60.1±11.3	41.2±10.2	35.6±9.2
IH-901	25	12	79.1±15.5	73.3±17.8	39.7±9.9	45.3±9.2
	250	12	81.2±18.5	57.5±17.4	41.3±13.8	44.8±10.2
Chlorpromazine	3	10	15.4±11.0*	1.3±1.2**	2.1±1.2**	4.8±2.3**

Each value represents the mean±SEM.
*$p<0.05$, **$p<0.01$; compared with vehicle treated group (student *t*-test).

Table III. Effect of IH-901 on pentobarbital-induced sleeping time in mice

Drug	Dose (mg/kg)	No. of animal	Sleeping time (min)	
			Onset	Duration
Vehicle	-	10	4.1±0.2	46.0±4.3
IH-901	25	8	5.5±0.8	52.3±4.8
	250	8	5.1±0.8	58.1±5.4
Chlorpromazine	3	8	6.2±0.5	67.8±3.1*

Each value represents the mean±SEM.
*$p<0.01$; compared with vehicle treated group (student *t*-test).

time in mice, when compared with vehicle group (Table III).

Effect on rotarod test

IH-901 (25 and 250 mg/kg) did not affect the motor coordination in mice for 4 hrs (Table IV).

Effects on PTZ-induced convulsions

No alterations in the PTZ-induced clonic seizure were

Table IV. Effect of IH-901 on rota-rod test in mice

Drug	Dose (mg/kg)	No. of animal	Number of mice which fell down			
			0.5 hr	1 hr	2 hr	4 hr
Vehicle	-	14	2	1	0	0
IH-901	25	14	1	1	0	0
	250	14	0	1	0	1
Chlorpromazine	3	10	8*	10*	10*	9*

*$p<0.01$; compared with vehicle treated group (Chi-square test).

Table V. Effect of IH-901 on pentylenetetrazole-induced convulsion in mice

Drug	Dose (mg/kg)	No. of animal	Convulsion		
			Onset time(min)	No. of convulsion	No. of death
Vehicle	-	13	3.9±2.6	13	13
IH-901	25	13	3.1±1.1	13	11
	250	13	5.1±3.4	13	12
Phenobarbital	100	8	-	0*	0*

Each value represents the mean±SEM.
*$p<0.01$; compared with vehicle treated group (Chi-square test).

Table VI. Effect of IH-901 on acetic acid-induced writhing syndrome in mice

Drug	Dose (mg/kg)	No. of animal	Number of writhing response
Vehicle	-	12	33.8±2.7
IH-901	25	12	33.0±3.9
	250	12	26.3±3.0
Aminopyrine	50 (s.c)	10	4.9±0.9*

Each value represents the mean±SEM.
*$p<0.01$; compared with vehicle treated group (student *t*-test).

observed in mice following oral administration of IH-901 (25 and 250 mg/kg) (Table V).

Effect on analgesic activity

As shown in Table VI, IH-901 (25 and 250 mg/kg) did not show any analgesic effects.

Effect on body temperature

For 4 hrs after oral treatment of IH-901 (25 and 250 mg/kg), no significant changes in the rectal body temperature were observed in mice (Table VII).

Table VIII. Effect on IH-901 on small intestinal peristaltic movement

Drug	Dose (mg/kg)	No. of animal	Rate of movement (%)
Vehicle	-	9	39.2±3.7
IH-901	25	9	41.3±7.4
	250	10	45.4±9.2

Each value represents the mean±SEM.

Effect on gastrointestinal system

Effect on intestinal propulsion

As shown in Table VIII, IH-901 (25 and 250 mg/kg) caused no observable effects on the intestinal motility in mice.

Effect on gastric acid secretion

No significant changes in volume, pH, free HCl concentration and total acidity of the gastric juice were observed in rats following oral administration of IH-901 at doses of 25 and 250 mg/kg (Table IX).

Effect on mean blood pressure and heart rate

As shown in Table X and XI, orally administered IH-901

Table VII. Effect of IH-901 on body temperature in mice

Drug	Dose (mg/kg)	No. of animal	Body temperature (°C)				
			before	0.5 hr	1hr	2hr	4hr
Vehicle	-	8	38.4±0.2	37.7±0.2	37.9±0.3	38.4±0.7	37.9±0.2
IH-901	25	8	38.2±0.1	37.0±0.4	37.8±0.5	38.2±0.3	38.4±0.2
	250	8	38.6±0.1	37.4±0.4	37.4±0.3	38.0±0.1	38.3±0.4
Chlorpro-mazine	3	8	38.1±0.2	35.9±0.7*	35.1±0.8**	34.8±0.9**	35.3±0.6**

Each value represents the mean±SEM.
*$p<0.05$, **$p<0.01$; compared with vehicle treated group (student *t*-test).

Table IX. Effect of IH-901 on gastric secretion in rats

Drug	Dose (mg/kg)	No. of animal	pH	Gastric vol. (ml)	Free HCl (mmol/L)	Total acidity (mmol/L HCl)
Vehicle	-	11	1.3±0.3	11.0±0.8	64.9±4.9	81.1±4.6
IH-901	25	11	1.2±0.1	10.1±0.6	77.4±9.1	95.1±8.2
	250	10	1.1±0.3	12.0±0.7	74.5±6.0	90.3±5.9

Each value represents the mean±SEM.

Table X. Effect of IH-901 on mean arterial blood pressure in rats

Drug	Dose (mg/kg)	No. of animal	Mean arterial blood pressure (mmHg)					
			before	0.5 hr	1 hr	1.5 hr	2 hr	3 hr
Vehicle	-	6	113.8±3.5	108.3±5.5	105.4±4.0	107.5±2.0	104.2±3.1	106.6±3.9
IH-901	25	6	117.5±2.7	115.0±6.7	112.0±6.5	112.0±7.2	112.0±6.0	106.5±5.3
	250	6	119.0±1.0	112.0±5.6	116.0±4.0	113.0±4.3	105.5±10.2	113.0±3.5
Hydrala-zine	50	6	117.6±2.4	87.2±4.3*	85.8±6.8*	91.3±4.3**	81.6±4.7**	83.8±5.2**

Each value represents the mean±SEM.
*$p<0.05$, **$p<0.01$; compared with vehicle treated group (student *t*-test).

Table XI. Effect of IH-901 on Heart rate in rats

Drug	Dose (mg/kg)	No. of animal	Heart rate					
			before	0.5 hr	1 hr	1.5 hr	2 hr	3 hr
Vehicle	-	6	420.5±9.8	413.5±21.8	383.3±32.1	376.3±26.5	357.0±26.3	375.5±24.9
IH-901	25	6	442.02±8.8	409.6±48.8	411.8±14.1	103.0±19.3	395.2±15.5	407.4±13.2
	250	6	388.0±4.6	371.4±27.0	396.4±28.3	410.0±24.6	375.4±31.4	366.2±19.8
Hydrala-zine	50	6	410.7±3.5	532.3±19.3*	528.1±21.0*	514.3±22.2*	507.7±20.2*	498.1±18.3*

Each value represents the mean±SEM.
*$p<0.01$; compared with vehicle treated group (student *t*-test).

Table XII. Effect of IH-901 on respiratory rate in rats

Drug	Dose (mg/kg)	No. of animal	Respiratory Rate (beats/min)					
			before	0.5 hr	1 hr	1.5 hr	2 hr	3 hr
Vehicle	-	7	83.4±3.6	74.2±3.2	77.5±2.1	79.7±4.5	77.1±4.7	87.8±8.0
IH-901	25	7	80.4±3.3	83.4±5.7	77.9±3.0	79.0±3.6	83.9±5.4	86.3±4.0
	250	8	88.7±4.2	80.7±3.2	77.6±3.8	80.5±4.7	77.1±5.3	88.5±6.4

Each value represents the mean±SEM.

(25 and 250 mg/kg) did not produce any significant changes in blood pressure and heart rate for 3 hrs after the drug administration.

Effect on respiratory rate of anesthetized rats

Orally administered IH-901 (25 and 250 mg/kg) did not show any effect in the respiratory rate, when compared with vehicle group (Table XII).

Effect on isolated smooth muscle

The additions of 25 μg /ml of IH-901 did not cause the relaxation or contraction of the smooth muscle. IH-901 at concentration of 25 μg/ml did not affect acetylcholine or histamine-induced log dose-response contractile activity (Fig. 1 and 2).

DISCUSSION

The purpose of the present study was to examine the pharmacologic properties of IH-901 to get some insight into the potential side effects on the central nervous, cardiovascular, gastrointestinal and the other organ systems, resulting from the secondary pharmacologic activity of high doses of the test substance.

At doses approximately 100 times higher than the anticipated clinical dose of IH-901, there were no obvious effects on the central nervous system, cardiovascular system, gastrointestinal system and respiratory system.

Based on these results, it was concluded that IH-901 did not induce any adverse effects in experimental animals.

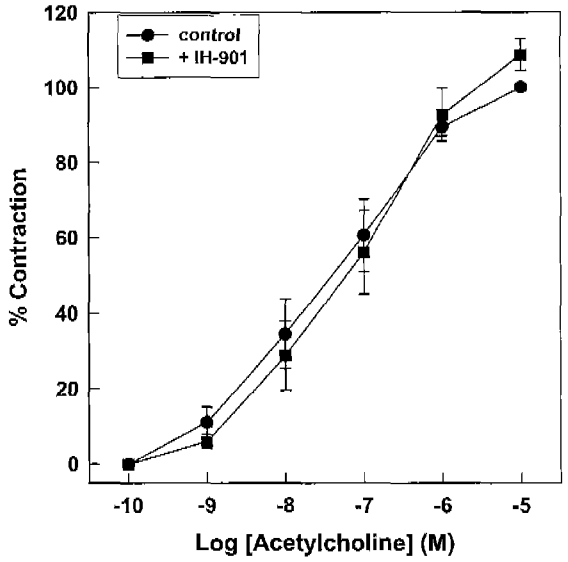

Fig. 1. Effect of IH-901 on acetylcholine (Ach)-induced contractions in isolated guinea pig ileum. Concentration-response curves of acetylcholine were obtained either in the absence (●) or presence of IH-901 25 μg/ml (■). To obtain concentration-response curves, acetylcholine was added cumulatively, and IH-901 was treated 5 min prior to acetylcholine addition. Values are mean ± SEM for 5 preparations.

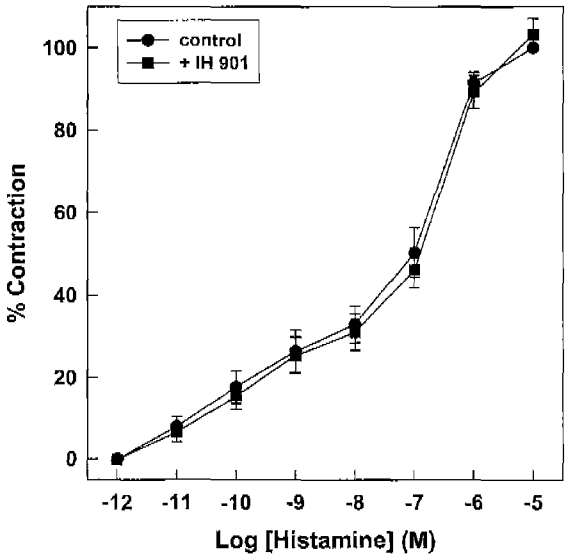

Fig. 2. Effect of IH-901 on histamine (His)-induced contractions in isolated guinea pig ileum. Concentration-response curves of histamine were obtained either in the absence (●) or presence of IH-901 25 μg/ml (■). To obtain concentration-response curves, histamine was added cumulatively, and IH-901 was treated 5 min prior to histamine addition. Values are mean ± SEM for 5 preparations.

ACKNOWLEDGEMENT

This research was supported by a grant from Central Research Institute, ILHWA Co. Ltd.

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