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General Pharmacology of ADP

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Abstract–General pharmacological properties of ADP, a new pharmaceutical composition, which contains a mixed water extract obtained from the mixture of Phellodendron cortex (*Phellodendron amurense*) and Anemarrhena rhizoma (*Anemarrhena asphodeloides*), as the active ingredients, were investigated in experimental animals administering orally and *in vitro* test system. ADP had no influences 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 150 and 1500 mg/kg. Gastric secretion of rats and intestinal motility of mice were not also influenced by the administration of ADP at doses of 150 and 1500 mg/kg, with the exception of the significant decrease of free HCl concentration at a dose of 1500 mg/kg in rats. ADP (150 and 1500 mg/kg) did not alter mean arterial blood pressure and heart rate in conscious rats. ADP given to anesthetized rats showed no effect on respiratory rate at the same doses. In *in vitro* experiments, ADP at the concentration of 150 mg/L did not show direct effect and inhibitory or augmentative action on histamine- or acetylcholine-induced contractions in the isolated ileum of guinea-pig. Taken together, these results indicate that ADP does not induce any adverse effects in experimental animals.

Keywords □ ADP, general pharmacology

INTRODUCTION

Benign prostate hyperplasia (BPH) is one of the prevailing diseases among at least 50% of aged males over fifty and estimated as a degenerative disease involving the enlargement of prostate gland due to intact androgen supply as the man gets old. In the early stage of forties, nodules can generate in the transition (grandula) and periurethral zones (stromal). The nodules formed in the transition zone continuously grow into the major part of the main mass of the prostate, in which case the central and peripheral zones are compressed and fibromuscular tissue develops between the BPH tissue. This progresses over several years and symptoms appear as the enlarged prostate obstructs the urinary track.

The recent therapies of BPH such as prostatectomy or removal of the dilative prostate using a laser beam are a temporary treatment that cannot inhibit lasting dilation of the prostate. Therapeutic drugs used against BPH are alpha-adrenergic antagonists inhibiting tone of the prostate or reducing androgen hormones production to prevent prostate dilation, but cannot be used in a continuous manner due to side effects.

Anemarrhena rhizoma (*Anemarrhena asphodeloides*) is reported to be effective for anti-inflammation, removal of fever, anti-diarrhea, diuresis, anti-lumbago and sedation. The main active components are known to be saponins including timosaponin, sarsasapogenin, and neogitogenin. (Ahn, 1998; Huang, 1999). Phellodendron cortex (*Phellodendron amurense*) is the bark of Phellodendron trees, which contains about 1.5-4.5% of aqueous alkaloids including berberine and phellodendrine. These components are known to act on jaundice, and to have antibacterial, antihypertensive, anti-inflammatory, and the CNS inhibitory effects. They are also effective on typhoid and gastrointestinal disease, and have the characteristics as astringent anti-inflammatory agents against gastroenteritis and abdominal pain (Ahn, 1998; Huang, 1999). However, a new pharmaceutical composition (ADP), which contains a mixed water extract obtained from the mixture of Phellodendron cortex and Anemarrhena rhizoma, as the active ingredients, provided a certain possibility to be used effectively for the treatment of BPH, since this composition reduces inflammation and potentiates the relaxation activity of contracted prostatic and urethral smooth muscle system in rats (Hong *et al.*, 2002).

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

MATERIALS AND METHODS

Animals

The animals used were male ICR mice (20-30 g), male Sprague-Dawley rats (250-300 g), and male Hartley guinea pigs (300-350 g) (Sam:TacN(SD)BR, Osan, Keonggi, Korea). Animals were housed in an acrylfiber cage in a controlled room (temperature 22±2°C, relative humidity 5±05%), were maintained on a 12-hr light/dark cycle and were given solid diet and tap water *ad libitum*. Animals were divided into groups at random.

Test Substance and Dose Determination

ADP was provided from Medvil Rescarch Lab. (Seoul, Korea). The 50% lethal dose (LD_{50}) of ADP was determined as more than 5,000 mg/kg in rats and the predicted clinical dose of ADP was 1-2 g/day/adult in human being (60 kg). Doses of 150 or 1500 mg/kg was applied *per oral* to mice and rats, after suspended in physiological saline. Control group of the mice was administered (0.1 mL/10 g) with physiological saline.

Drugs

Pentylenetetrazole, aminopyrine, chlorpromazine, hydralazine, 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 highest grade available.

Effects on General Behavior

The methods used were based on that described by Irwin (1968). Mice were administered orally with ADP (150 and 1500 mg/kg), and were observed at 0.5, 1, 2 and 4 hrs after the drug administration.

Effect on Central Nervous System

Spontaneous Locomotor Activity

Mice were orally administered with ADP or vehicle. Each mouse was then placed in an activity cage with an automatic recording device (AM1051, Benwick Electronics, Benwick, UK), and activity counts for 5 min were recorded 0.5, 1, 2, 3 and 5 hr after the drug administration. Data were shown as a percentage of the value measured before the drug administration.

Pentobarbital-Induced Sleeping Time

Mice were orally administered with ADP or vehicle. One hr after, 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.

Rotarod Test

Mice were orally administered with ADP or vehicle and were subjected to the rotarod tests 0.5, 1, 2, 3 and 5 hr after the drug administration. The mice were placed on a 3 cm diameter rod (Daejong Ins.) 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).

Pentylenetetrazol (PTZ)-Induced Convulsion

PTZ (110 mg/kg) was injected subcutaneously 1 hr after the administration of test drugs. 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.

Analgesic Activity

Each mouse was injected with 0.8% acetic acid (0.1 mL/10 g. i.p.) 1 hr after the administration of ADP or vehicle, and was placed immediately in an observation cage. Ten min after the injection of acetic acid, the numbers of writhing episodes during the subsequent 10-min period were counted (Collier *et al.*, 1968).

Body Temperature

Body temperature was measured rectally using an electrothermometer (Thermalert TH-5, Physitemp, USA). ADP or vehicle was administered orally to male mice (22-25 g) with stable rectal temperature and rectal temperatures were measured 0.5, 1, 2, 3 and 5 hr after the drug administration.

Effect on Gastrointestinal System

Intestinal Propulsion

The mice fasted overnight were administered orally with ADP or vehicle. Fifty min 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 min 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 ligated and then ADP was intraduodenally given. Five hr after the abdomen closed, the rats were sacrificed and stomach 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 Sprague-Dawley 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, 3 and 4 hr after single oral administration of ADP.

Effect on Respiratory Rate

ADP 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 90 min. The number of respiration for 1 min was measured at the time of 0.5, 1 and 1.5 hr after the single oral administration of ADP.

Effect on Smooth Muscle

Agonist-induced Contractions in Isolated Guinea Pig Ileum

Segments of myenteric 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 preparations 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 preparations were equilibrated for about 60 min before each 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 test drug on smooth muscle, the preparations were exposed to the test drug for 5 min, and then agonists (acetylcholine or histamine) were 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 AND DISCUSSION

Effect on General Behavior

Oral administration of ADP at doses of 150 and 1500 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 Locomotor Activity

ADP at the dose of 150 mg/kg did not show any significant change in spontaneous locomotor activity for 5 hrs. However, oral administration of 1500 mg/kg ADP showed inhibition of locomotor activity, but it was not significant change, when compared to the vehicle-treated control group (Table II).

Effect on Pentobarbital-Induced Sleeping Time

Orally administered ADP (150 and 1500 mg/kg) did not show any significant change of the pentobarbital-induced sleeping time in mice, when compared with vehicle group (Table III).

Effect on Rotarod Test

ADP (150 and 1500 mg/kg) did not affect the motor coordination in mice for 5 hrs (Table IV).

Effects on PTZ-induced Convulsions

No alterations in the PTZ-induced clonic seizure were

Table I. Effect of ADP 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 reflix	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: ADP 150 mg/kg, C: ADP 1500 mg/kg

Table II. Effect of ADP on spontaneous locomotor activity in mice

Drug	Dose (mg/kg, p.o)	No. of animal	Activity (% of Prevalue)				
			0.5 hr	1 hr	2 hr	3 hr	5 hr
Vehicle	–	10	101.6±26.0	62.5±11.9	77.1±14.8	58.0±12.0	48.6±9.7
ADP	150	10	104.8±24.9	72.5±16.5	50.2±12.3	60.9±15.8	54.9±13.3
	1500	10	51.1±9.0	41.2±8.1	36.8±6.0	31.1±7.6	32.4±4.2
chloropromazine	3	10	18.4±13.0*	1.2±1.1**	2.0±1.4**	4.6±2.9**	7.7±5.5**

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

observed in mice following oral administration of ADP (150 and 1500 mg/kg) (Table V).

Effect on Analgesic Activity

As shown in Table VI, ADP (150 and 1500 mg/kg) did not show any analgesic effects.

Effect on Body Temperature

For 5 hrs after oral treatment of ADP (150 and 1500 mg/kg), no significant changes in the rectal body temperature were observed in mice (Table VII).

Effect on Gastrointestinal System

Effect on Intestinal Propulsion

As shown in Table VIII, ADP (150 and 1500 mg/kg) caused no observable effects on the intestinal motility in mice.

Table III. Effect of ADP on pentobarbital sleeping time in mice

Drug	Dose (mg/kg)	No. of animal	Sleeping time (min)	
			Onset	Duration
Vehicle	–	12	6.3±0.6	39.7±2.0
ADP	150	12	7.4±0.4	35.1±4.1
	1500	12	7.6±0.7	43.0±3.5
Chlorpromazine	3	12	6.0±0.3	59.8±3.4*

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

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

Drug	Dose (mg/kg)	No. of animal	Number of mice which fell down				
			0.5 min	1 hr	2 hr	3 hr	5 hr
Vehicle	–	10	0	0	0	0	0
ADP	150	10	0	0	0	0	0
	1500	10	0	1	0	0	0
Chlorpro-mazine	3	8	6*	8*	8*	7*	7*

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

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

Drug	Dose (mg/kg)	No. of animal	Convulsion		
			Onset time (sec)	No. of convulsion	No. of death
Vehicle	–	10	199.4±19.2	10	10
ADP	150	10	268.1±40.7	8	8
	1500	10	214.4±15.7	10	9
Phenobarbital	100	8	–	0*	0*

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

Effect on Gastric Acid Secretion

No significant changes in volume, pH and total acidity of the gastric juice were observed in rats following oral administration of ADP at doses of 150 and 1500 mg/kg. However, 1500 mg/kg of ADP showed significant decrease in free HCl concentration of the gastric juice (Table IX).

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

Drug	Dose (mg/kg)	No. of animal	Number of writhing response
Vehicle	–	10	21.8±5.7
ADP	150	10	22.0±3.5
	1500	10	19.2±3.3
Aminopyrine	50 (s.c)	10	1.9±0.9*

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

Table VIII. Effect on ADP on small intestinal peristaltic movement

Drug	Dose (mg/kg)	No. of animal	Rate of movement (%)
Vehicle	–	10	35.2±2.0
ADP	150	10	36.3±2.4
	1500	10	38.7±2.9

Each value represents the mean±SEM.

Effect on Mean Blood Pressure and Heart Rate

As shown in Table X and XI, orally administered ADP (150 and 1500 mg/kg) did not produce any significant changes in blood pressure and heart rate for 4 hrs after the drug administration, except the slight reduction in both parameters at 4 hr at a dose of 1500 mg/kg.

Effect on Respiratory Rate of Anesthetized Rats

Thirty min after the oral administration of ADP (150 and 1500 mg/kg), the respiratory rate of rats was reduced with the anesthesia. However, the test drug did not show any effect in the

Table VII. Effect of ADP on body temperature in mice

Drug	Dose (mg/kg)	No. of animal	Body temperature (°C)					
			before	0.5 min	1 hr	2 hr	3 hr	5 hr
Vehicle	–	12	36.9±0.1	36.8±0.2	36.8±0.2	36.6±0.3	36.2±0.2	36.2±0.2
ADP	150	12	36.8±0.1	36.9±0.1	36.9±0.1	36.5±0.3	36.2±0.2	36.3±0.2
	1500	12	36.7±0.1	36.8±0.1	36.8±0.2	36.4±0.2	36.2±0.2	36.3±0.2
Chlorpro-mazine	3	12	37.1±0.1	34.2±0.7*	33.3±0.8*	33.3±0.9*	34.3±0.6*	35.0±0.3*

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

Table IX. Effect of ADP 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	–	6	1.3±0.1	13.3±2.5	126.3±11.8	159.3±14.8
ADP	150	6	1.3±0.1	9.7±1.5	117.3±17.4	146.3±18.1
	1500	6	1.6±1.2	12.4±1.2	89.0±7.3*	151.7±11.2

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

Table X. Effect of ADP 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	4 hr
Vehicle	–	6	107.7±2.1	110.81±2.8	115.5±4.3	115.4±4.5	111.7±4.5	105.8±4.5	103.9±3.1
ADP	150	6	105.0±4.2	101.0±3.6	104.7±5.0	98.8±5.2	97.0±5.1	97.3±6.4	101.3±4.3
	1500	6	105.7±4.2	104.8±3.9	104.8±4.0	109.1±3.4	105.8±4.3	102.5±3.4	92.0±4.5
Hydralazine	50	6	107.6±2.6	80.2±4.3**	75.8±6.8**	80.0±6.4**	81.6±4.7**	83.8±5.2**	87.8±4.9**

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

Table XI. Effect of ADP 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	4 hr
Vehicle	–	6	398.3±28.9	378.5±27.9	366.8±28.4	392.7±29.2	382.6±26.4	387.9±27.3	407.6±25.2
ADP	150	6	399.6±22.0	388.7±23.8	425.2±25.1	421.5±28.3	416.5±31.2	421.4±38.6	404.7±28.4
	1500	6	391.2±18.8	397.2±22.7	373.6±16.6	386.0±23.9	378.5±17.5	388.7±21.2	354.3±25.0
Hydralazine	50	6	379.7±13.0	512.8±21.3**	510.1±24.0**	494.3±26.2*	488.7±23.5*	478.2±15.9*	501.3±15.0**

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

Table XII. Effect of ADP 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
Vehicle	–	7	83.7±4.0	50.9±2.3	52.7±4.4	62.1±3.2
ADP	150	6	84.0±4.4	51.5±2.1	52.5±2.4	58.6±4.2
	1500	6	80.3±3.3	57.0±1.5	47.0±2.4	58.5±1.9

Each value represents the mean±SEM.

respiratory rate, when compared with vehicle group (Table XII).

Effect on Isolated Smooth Muscle

The additions of 150 and 1500 μg/mL of ADP did not cause the relaxation or contraction of the smooth muscle. ADP at concentration of 150 μg/mL did not affect acetylcholine or histamine-induced log dose-response contractile activity. However, 1500 μg/mL of ADP showed noncompetitive inhibition on acetylcholine- or histamine-induced contractile activity on the smooth muscle, since these agonists-induced maximal responses were depressed at this concentration (Fig. 1 and 2).

The purpose of the present study was to examine the pharmacologic properties of ADP in an attempt to gain 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 agent.

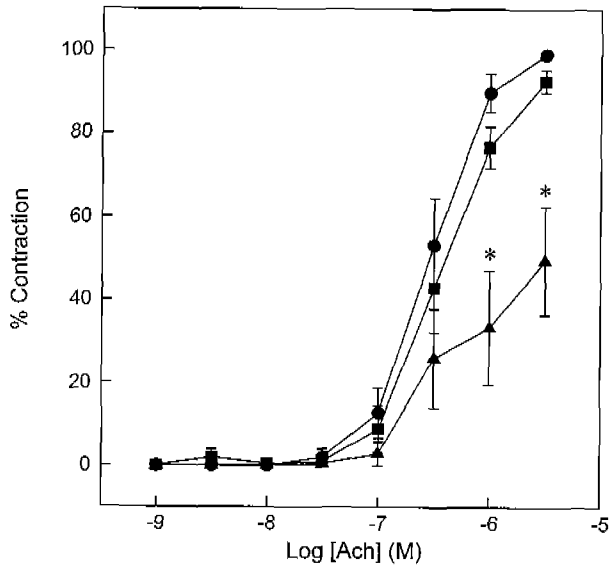

Fig. 1. Effect of ADP on acetylcholine (Ach)-induced contractions in isolated guinea pig ileum. Concentration-response curves of acetylcholine were obtained either in the absence (●) or presence of ADP 150 μg/mL (■) and 1500 μg/mL (▲). To obtain concentration-response curves, acetylcholine was added cumulatively, and ADP was treated 5 min prior to acetylcholine addition. Values are means±SEM for 5 preparations. *p<0.01; compared with acetylcholine (student *t*-test).

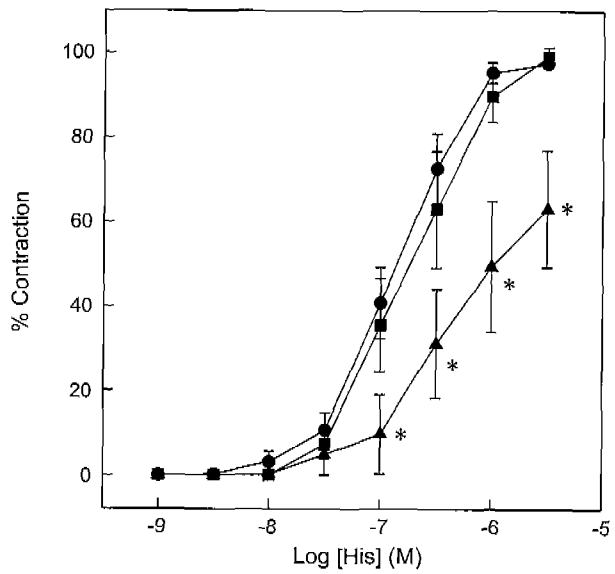

Fig. 2. Effect of ADP on histamine (His)-inducontractions in isolated guinea pig ileum. Concentration-response curves of histamine were obtained either in the absence (●) or presence of ADP 150 μg/mL (■) and 1500 μg/mL (▲). To obtain concentration-response curves, histamine was added cumulatively, and ADP was treated 5 min prior to histamine addition. Values are means± SEM for 6 preparations. *p<0.05; compared with histamine (student *t*-test).

At doses approximately 100 times higher then the anticipated clinical dose of ADP, there was no obvious effects on the central nervous system, cardiovascular system, gastrointestinal system and respiratory system. In the smooth muscle, however, ADP produced an inhibitory effect on the contractions induced by acetylcholine and histamine in the isolated ileum of the guinea pig at a concentration of 1500 μg/mL, suggesting the pharmacological action of ADP on the autonomic nervous system at high dose.

Taken together, our results suggest that ADP does not appear to have significant general pharmacological activities.

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