CHEMICAL & PHARMACEUTICAL BULLETIN

Vol. 23, No. 8 August 1975

Regular Articles

[Chem. Pharm. Bull. 23(8)1639—1645(1975)]

UDC 547.435.09 : 615.31.076.9

Ganglion Blocking Activity of Amidinothiocholine Derivatives[1]

Hikaru Ozawa,[2] Tai Soon Cho[2a] and Kazunobu Sugawara[2]

Pharmaceutical Institute, Tohoku University[2]

(Received March 29, 1974)

Some amidinothiocholine derivatives have been prepared and tested for the ganglion blocking activity, as compared with that of hexamethonium. Among them, most potent ganglionic blocking compound was *S*-(2-trimethylaminoethyl)-1′-ethylisothiuronium which was more potent than hexamethonium on the cat superior cervical ganglion, the guinea-pig hypogastric nerve–vas deferens preparation and the cat and rat blood pressure. Other compounds, also, had the ganglionic blocking action, but were not so potent as hexamethonium.

In a study concerning the structure-activity relationship of a series of amidino compounds, the authers have studied the pharmacological actions of 2-aminoethylisothiuronium derivatives[3] and observed that amidinothiocholine, one of *N*-trimethyl derivatives of 2-aminoethylisothiuronium, as well as acetylcholine (ACh), possessed a stimulating action in sympathetic ganglion. Also it was shown that it possessed only a weak muscarinic action in cholinergic nerve ending.[4]

Amidinothiocholine is a compound obtained by substituting thiourea for acetoxy group of ACh. Authors synthesized derivatives of amidinothiocholine replaced with other alkyl-groups to each N position and studied the pharmacological actions. Among them, *N*′-methyl derivative, *S*-(2-trimethylaminoethyl)-1′-methylisothiuronium (MTMA) and *N*′-ethyl derivative, *S*-(2-trimethylaminoethyl)-1′-ethylisothiuronium (ETMA) had a stimulating action and a blocking action of autonomic ganglion, respectively.[3b,3c]

In the present paper, the authors investigated and evaluated the ganglionic blocking actions of newly synthetized compounds, *S*-(2-triethylaminoethyl) isothiuronium (TEAEI), *S*-(2-triethylaminoethyl)-1′-methylisothiuronium (MTEAI) and 2-(2-triethylaminoethylthio)-Δ^2-imidazoline (ETEAI), comparing with a typical ganglion blocking agent, hexamethonium.

Methods and Materials

1. Cervical Sympathetic Ganglion of the Cat——Cats (2—4 kg) of both sexes were anaesthetized with 60 mg/kg α-chloralose intravenously. After tracheal cannulation, cats were prepared for recording. The

1) A part of this work was reported at the 92th Annual Meeting of Pharmaceutical Society of Japan, Osaka, April 1972.

2) Location: *Aobayama, Sendai*; *a*) Present adress: *Department of Pharmacology, Yonsei University College of Medicine, Shinchondong, Seoul, Korea.*

3) *a*) H. Ozawa and T.S. Cho, *Yakugaku Zasshi*, **91**, 624 (1971); *b*) *Idem, ibid.*, **91**, 822 (1971); *c*) *Idem, Folia pharmacol. japon.*, **68**, 19 (1972).

4) H. Ozawa and Y. Hara, *Folia pharmacol. japon.*, **64**, 467 (1968).

contraction of one nictitating membrane was recorded on a kymograph with an isotonic writing lever. The contractions were induced by the stimulation of the ipsilateral pre- and post-ganglionic cervical sympathetic nerves. The electrical stimulation was applied at a frequency of 20 cps with 1 msec duration and at 5 V for preganglionic fiber and 10 cps, 1 msec, 10 V for postganglionic fiber. The direct injection of drugs to ganglion was carried out as described by Trendelenburg.[5] In this method, a polyethylene cannula was inserted into the lingual artery, and drug was injected retrogradely into the carotid artery through the cannula. During intraarterial injection, the external carotid artery was occluded with a clip. The drugs were usually injected in a volume of 0.3 ml.

2. **Guinea-pig Isolated Hypogastric Nerve-Vas Deferens Preparation**——After the vas deferens was dissected together with the hypogastric nerve from guinea-pig, the preparation was suspended in a 10 ml bath containing Tyrode solution at 32 ° and aerated with 95% O_2+5% CO_2. The contraction of the vas deferens was recorded on a kymograph with an isotonic writing lever. The electrical stimulation to the preganglionic fiber of the hypogastric nerve was applied every 3 min at a frequency of 20 cps with 0.1 msec duration and 7 V for 3 sec.

3. **Cat and Rat Blood Pressure**——Cats (2—4 kg) and rats (200—300 g) were anaesthetized with 60 mg/kg α-chloralose intravenously and with 1.4 g/kg urethane subcutaneously, respectively. Blood pressure was recorded from the right carotid artery and the injections of drugs were made into a femoral vein for cat and a juglar vein for rat.

4. **Gastrointestinal Propulsion in Mice**——Mice (18—22 g) fasted for 24 hr were used. After 10 min of subcutaneous injections of test compounds, 20% $BaSO_4$ solution was administered orally. After 30 min the small intestine was isolated and the ratio of the length of $BaSO_4$ propulsion for that of the intestine from the pyloric region to the cecum was calculated.

5. **Antiulcer Action and Antigastric Secretion**——Male Wistar rats (200—300 g) were starved for 48 hr in the individual cages with access to water. Under ether anaesthesia, the abdomen was incised and the pyloro-duodenal junction was ligated, as described by Shay, *et al.*[6] Four and 19 hr after the operation they were killed and their stomachs were removed. Gastric contents were collected and centrifuged at 10000 rpm for 10 min, and the volume of supernatant solution and the pH value were measured. Total acidity and the free HCl were titrated with 0.1 N NaOH by using phenolphthalein and Töpfer's reagents as indicators, respectively.[7] Test compounds dissolved in 0.9% saline was administered intraperitoneally with pylorus ligation.

For the determination of anti-ulcerous effect, the stomach was cut open along the greater curvature and stretched out. As a criterion of the degree of ulceration, the size of ulcers as shown Table I was used. With this scoring system an ulcer index (UI) is obtained for each animal that gave a satisfactory objective evaluation of ulcerations. The UI of a single rat is sum of the scores for each ulcer.

Table I. Table of Ulcer Score

Group	Diameter of ulcer (mm)	Score
1	0— 2	1
2	2— 5	5
3	5—10	10
4	10—	20
5	perforation	20

6. **Acute Toxicity**——Mice (18—22 g) were used and the test compounds were injected subcutaneously, intraperitoneally or intravenously. For the subcutaneous and intraperitoneal injections, the method of Litchfield and Wilcoxon[8] was used, and LD_{50} was calculated from the lethal rate of the mice at 72 hr after the injection. For the intravenous injection, the "up and down" method was used.

Materials——Amidinothiotriethylcholine Derivatives: 1-Bromoethane-2-triethylammonium bromide (I) was obtained from ethylene dibromide and triethylamine by Vidal's method.[9] The mixture of (I) and thiourea derivatives was melted at 100—150° for 30—40 min. The crystallized residue (II) after gassed was recrystallized from *n*-propanol and ethanol (Chart 1).

5) U. Trendelenburg, *Federation Proc.*, **18**, 1001 (1959).
6) H. Shay, S.A. Komarov, S.S. Fels, D. Meranze, M. Gruenstein, and H. Siplet, *Gastroenterology*, **5**, 43 (1945).
7) J.O. Keyriläinen and M.K. Passonen, *Acta Pharmacol. et Toxicol.*, **13**, 22 (1957).
8) J.T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exptl. Therap.*, **96**, 99 (1949).
9) F. Vidal, *J. Org. Chem.*, **24**, 680 (1959).

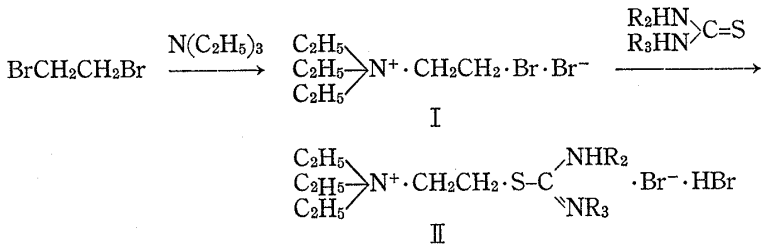
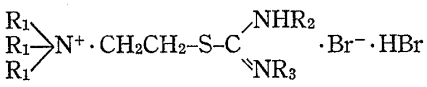


Chart 1

TABLE II. Chemical Structures of Test Compounds



Abbreviation	R_1	R_2	R_3	mp (°C)
ETMA	CH_3	C_2H_5	H	206
TEAEI	C_2H_5	H	H	178 —181
MTEAI	C_2H_5	CH_3	H	174.5—176.5
ETEAI	C_2H_5	$-CH_2CH_2-$		219 220 (decomp.)

ETMA : *S*-(2-trimethylaminoethyl)-1′-ethylisothiuronium
TEAEI : *S*-(2-triethylaminoethyl)isothiuronium
MTEAI : *S*-(2-triethylaminoethyl)-1′-methylisothiuronium
ETEAI : 2-(2-triethylaminoethylthio)-Δ^2-imidazoline

These compounds were identified by melting point, ultimate analysis and NMR. The chemical structures and melting points of test compounds were shown in Table II.

Other drugs used in these experiments were: *dl*-norepinephrine hydrochloride, l-epinephrine hydrochloride, acetylcholine chloride (ACh), 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP), hexamethonium bromide (C_6), potassium chloride (KCl), *l*-nicotine-*d*-bitartrate.

Results

1. Effects on Cervical Sympathetic Ganglion of the Cat

The contraction of the nictitating membrane produced by the electrical stimulation on preganglionic fiber of the cervical sympathetic nerve was suppressed by the intraarterial injections of test compounds. But the contraction produced by the electrical stimulation on postganglionic fiber was not influenced by these compounds. As an example, suppression by ETMA (0.1 mg), MTEAI (0.5 mg) and C_6 (0.5 mg) was shown in Fig. 1. The contraction of the nictitating membrane produced by DMPP (5 μg) was suppressed with these test compounds, but that induced by KCl (4 mg) was scarcely affected with them. The contraction of the nictitating membrane which was also produced by the intraarterial injection of epinephrine (20 μg) without occluding the external carotid artery was not affected with these compounds.

2. Effects on the Guinea-pig Isolated Hypogastric Nerve-Vas Defenens Preparation

The contraction of the vas deferens produced by the electrical stimulation on preganglionic fiber of the hypogastric nerve was suppressed by test compounds (10^{-6}—10^{-5} g/ml) and the action was reversible. The experimental examples were shown in Fig. 2. But the contraction of the vas deferens produced by the transmural electrical stimulation of postganglionic fiber was not suppressed even at a concentration of 10^{-4} g/ml.

In order to study the effects of test compounds on the hypogastric ganglion, an experiment was carried out using the compartment of the ganglion separated from that of the vas deferens with the segregated plate.[10] Although each compound administered in the compartment

10) H. Ozawa and F. Abe, *Folia pharmacol. japon.*, **70**, 727 (1974).

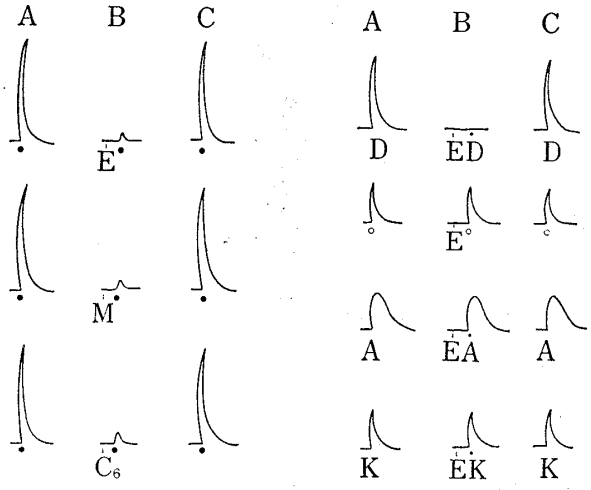


Fig. 1. Effects of ETMA, MTEAI and C_6 on the Nictitating Membrane Contraction in Anaesthetized Cat

Drugs were injected intraarterially. A: control response, B: drug effect, C: recovery in 10 min ●: electrical stimulation to preganglionic superior cervical nerve (20 cps, 1 msec, 5 V) ○: electrical stimulation to postganglionic superior cervical nerve (10 cps, 1 msec, 10 V) E: ETMA 0.1 mg M: MTEAI 0.5 mg C_6: hexamethonium 0.5 mg D: DMPP 5 μg A: epinephrine 20 μg K: KCl 4 mg.

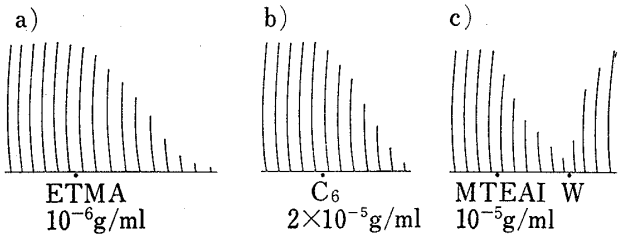


Fig. 2. Effects of ETMA, MTEAI and C_6 on the Isolated Guinea-pig Hypogastric Nerve-Vas Deferens Preparation

Electrical stimulation to preganglionic fiber was applied at 20 cps, 0.1 msec, 7 V. Drugs were administered at dots. a) ETMA 10^{-6} g/ml, b) C_6 2×10^{-5} g/ml, c) MTEAI 10^{-5} g/ml W: washing out.

of the vas deferens scarcely had effects on the contraction of the vas deferens produced by the electrical stimulation to preganglionic fiber of the hypogastric nerve, that administered in the compartment of the ganglion inhibited the contraction.

3. Effects on Cat and Rat Blood Pressure

In anaesthetized cats and rats, test compounds (0.3—30 mg/kg, *i.v.*) produced a fall in the blood pressure. In the rat, as shown in Fig. 3, the pressor actions of DMPP (0.1 mg/kg, *i.v.*) were suppressed by ETMA (10 mg/kg, *i.v.*) and MTEAI (30 mg/kg, *i.v.*). The pressor actions of epinephrine and norepinephrine (10 μg/kg, *i.v.*) were not affected by these compounds. Other test compounds also possessed these actions but the activities were weaker than those of ETMA and MTEAI.

In cat blood pressure, the pressor actions of ACh (0.3 mg/kg, *i.v.*) in the presence of atropine (2 mg/kg, *i.v.*), of nicotine (50 μg/kg, *i.v.*), or of bilateral carotid occlusion for 30 sec were almost completely suppressed by the intravenous injections of test compounds. The pressor action of epinephrine (10 μg/kg, *i.v.*), however, was not affected. Furthermore, the

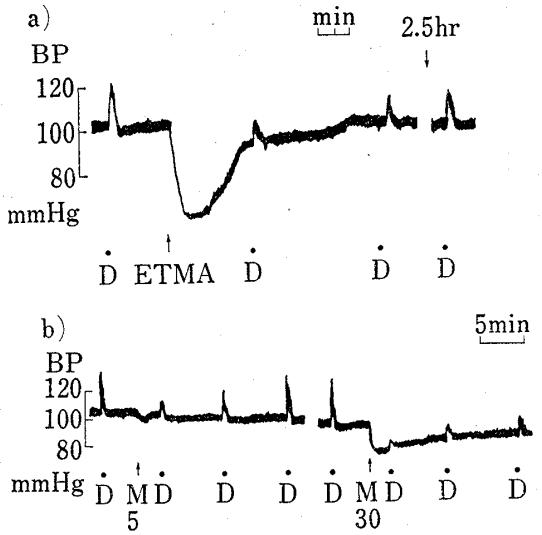


Fig. 3. Effects of ETMA and MTEAI on the Pressor Response Induced by DMPP (D: 0.1 mg/kg, *i.v.*) in Anaesthetized Rats

a) effect of ETMA (10 mg/kg, *i.v.*)
b) effect of MTEAI (M: 5 and 30 mg/kg, *i.v.*)

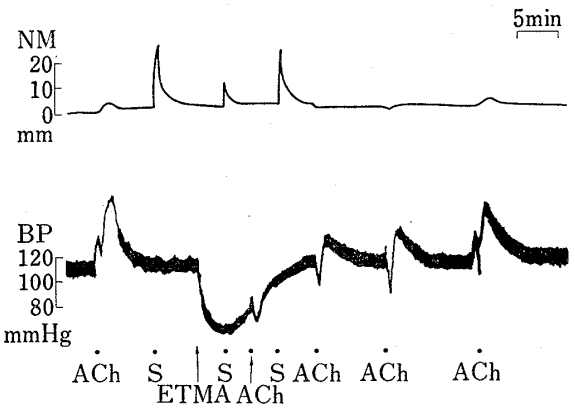


Fig. 4. Effect of ETMA (1 mg/kg, *i.v.*) on Blood Pressure and Nictitating Membrane in Atropinized (2 mg/kg, *i.v.*) Cat

upper trace: nictitating membrane lower trace: blood pressure S: electrical stimulation of preganglionic superior cervical sympathetic nerve (20 cps, 1 msec, 1.8 V, for 15 sec) ACh: ACh 0.3 mg/kg, *i.v.*

contraction of the nictitating membrane produced by the electrical stimulation of the cervical preganglionic fiber was suppressed by these compounds. The experimental examples of ETMA and MTEAI were shown in Fig. 4—7.

4. Effects on Gastrointestinal Propulsion in Mice

As shown in Table III, gastrointestinal propulsion was inhibited by test compounds and the action of ETMA was equivalent to that of C_6.

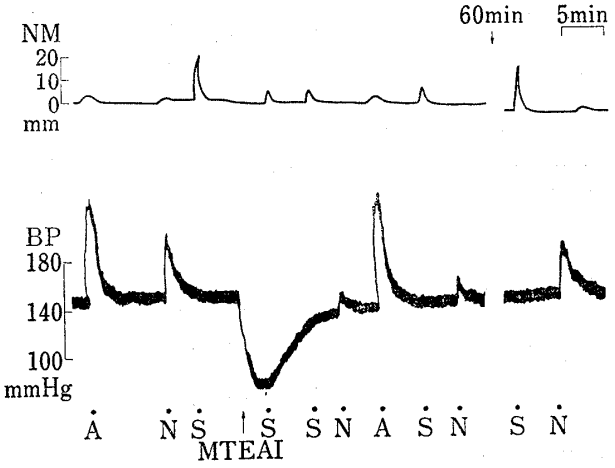


Fig. 5. Effect of MTEAI (5 mg/kg, *i.v.*) on Blood Pressure and Nictitating Membrane in Anaesthetized Cat

upper trace: nictitating membrane lower trace: blood pressure S: electrical stimulation of preganglionic superior cervical sympathetic nerve (20 cps, 1 msec, 1.8 V, for 15 sec) A: epinephrine 10 μg/kg *i.v.* N: nicotine 50 μg/kg *i.v.*

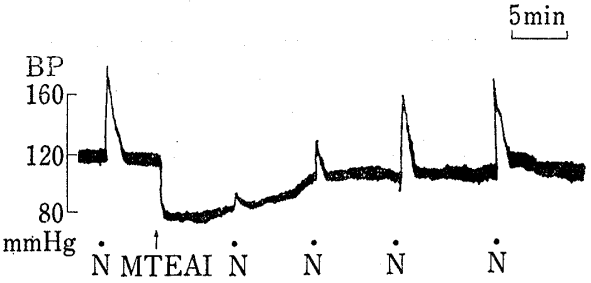


Fig. 6. Effect of MTEAI (5 mg/kg, *i.v.*) on the Pressor Response Induced by Nicotine in Anaesthetized Cat

N: nicotine 50 μg/kg *i.v.*

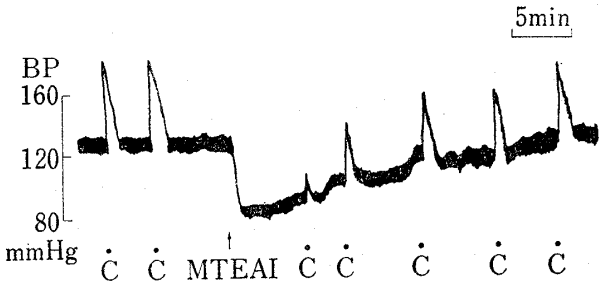


Fig. 7. Effect of MTEAI (5 mg/kg, *i.v.*) on the Pressor Response to Carotid Occlusion in Anaesthetized Cat

C: bilateral carotid occlusion for 30 sec

TABLE III. Effects of Test Compounds on Gastrointestinal Propulsion in Mice

	ED_{50} [a]
ETMA	3.7 mg/kg
TEAEI	73.5 mg/kg
MTEAI	63.8 mg/kg
ETEAI	46.3 mg/kg
C_6	3.8 mg/kg

a) Dose that produced 50% inhinition of $BaSO_4$ propulsion in the intestine

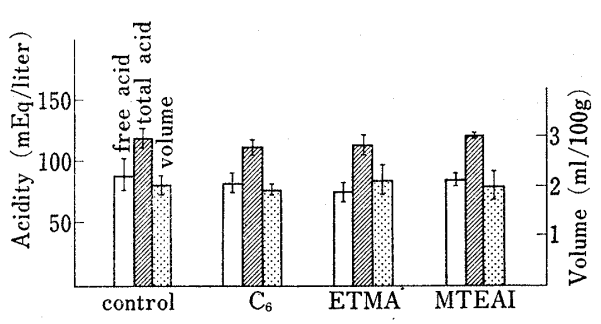


Fig. 8. Effects of C_6, ETMA and MTEAI on Gastric Secretion in the Stomach of Shay Rat (4 hr period)

As control, 0.9% saline was injected. Vertical bars represent standard errors.

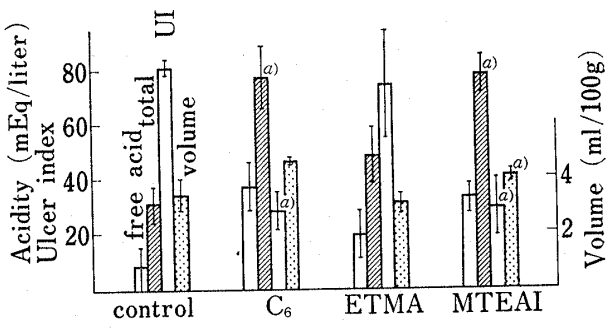


Fig. 9. Effects of C_6, ETMA and MTEAI on Gastric Secretion and Ulcer Formation in the Stomach of Shay Rat (19 hr period)

As control, 0.9% saline was injected. Vertical bars represent standard errors. *a)* $p<0.05$

5. Antiulcer Action and Antigastric Secretion

As shown in Fig. 8 and 9, the free acid, total acid, volume and UI measured from rat stomach 4 hr and 19 hr after the operation were scarcely influenced by test compounds. In rat 19 hr after the operation, however, only C_6 and MTEAI showed the antiulcer action and the increase of the total acid.

6. Acute Toxicity

The LD_{50} of the test compounds was shown in Table IV. The toxic symptoms were the extremities ataxia and the respiratory paralysis.

TABLE IV. Acute Toxicity in Mice (mg/kg)

	i.v.[a]	*i.p.*[b]	*s.c.*[b]
ETMA	81.8	95 (80.5—112.1)	102 (90.3—115.3)
TEAEI	48.8	90 (81.8— 99.9)	99 (92.8—105.6)
MTEAI	99.3	148 (140.9—156.2)	172 (163.5—180.9)
ETEAI	53.9	75 (70.9— 80.3)	109 (101.8—116.7)
C_6	26.3	86 (76.0— 96.9)	189 (173.0—223.0)

a) up and down method
b) Litchfield-Wilcoxon's method
parenthesis: 95% confidence limits

Discussion

In the present paper the ganglionic blocking actions of newly synthesized three compounds and ETMA, the mechanism of which was reported,[3c] were evaluated comparing with C_6, a typical ganglion blocker.

A marked hypotensive action was observed by the intravenous injection of all test compounds in cats and rats. This action would not be due to muscarine-like action because it was not influenced by atropine. Also, since these compounds did not show any inhibitory action to the pressor of norepinephrine, it would not be due to adrenolytic action. The suppression of the pendular movements of rabbit isolated intestine produced by the stimulation of mesentric nerve was not affected by test compounds. Therefore, it is possible that these hypotensive actions might not be due to the blocking action of the sympathetic nerve ending. On the other hand, it is obvious that each compound possesses the ganglionic blocking action because of the following results: 1) the suppression of the pressor responses induced by ganglion stimulants, carotid occulusion and ACh in the presence of atropine, 2) the suppression of the contraction of the guinea-pig isolated vas deferens induced by the electrical stimulation of the preganglionic fiber of the hypogastric nerve, and 3) the suppression of the gastrointestinal propulsion in mice.

In the experiments of cat's cervical ganglion, the contractions of the nictitating membrane induced by the stimulation of preganglionic fiber of the cervical sympathetic nerve and by the intraarterial injection of DMPP were suppressed by the intraarterial injections of test compounds, but those induced by the stimulation of postganglionic fiber and by the intraarterial injection of epinephrine were not. Also, the contraction of the nictitating membrane induced by an intraarterial injection of K^+ was not affected by these compounds. It would be considered, therefore, that the site of action of these compounds is postsynaptic cholinoceptive site. Nictitating membrane did not contract by intravenous injection of K^+ (4 mg) in this

experiment. It is possible to consider that intraarterial injection of K^+ (4 mg/kg) affected ganglion directly.

The effect of ETMA was 10—15 times that of C_6 and MTEAI was equal to C_6, and TEAEI and ETEAI were weaker than C_6 on the cat cervical ganglion, the isolated guinea-pig hypogastric nerve-vas deferens preparation and the pressor response induced by ganglion stimulant. On the gastrointestinal propulsion test in mice, the effect of ETMA was almost equal to that of C_6, and the following order was shown: ETEAI>MTEAI>TEAEI. Furthermore, the free acid, total acid, volume and UI measured from rat stomach were scarcely affected by these test compounds.

From the results described above, it is concluded that amidinothiocholine derivatives would be useful as a ganglionic blocking agent from the following points: 1) the general pharmacological action except the effect on the ganglion is very weak, 2) these compounds act on the ganglion and the efficacy is equal to or greater than C_6, 3) furthermore, the acute toxicities of them are relatively weaker than C_6.

Acknowledgement The authors wish to thank Miss K. Shikama for her technical assistance.