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THE INHIBITORY ACTION OF TWO THROMBIN INHIBITORS (TI-189 AND TI-233) ON THE CONTRACTILE RESPONSES TO 5-HYDROXYTRYPTAMINE AND PROSTAGLANDIN ENDOPEROXIDE ANALOGUE (U-44069) IN ISOLATED VASCULAR STRIPS

WILLIAM K.W. HO*, K. NAKAO & S. SHIBATA*

Department of Pharmacology*, School of Medicine, University of Hawaii, Honolulu, Hawaii, U.S.A. and Central Research Laboratory, Mitsubishi Chemical Ind, Yokohama, Japan

1 Two arginine derivatives that were developed as thrombin inhibitors (TI-189 and TI-233) selectively inhibited the 5-hydroxytryptamine (5-HT)-induced contraction of rabbit aortic strips in a competitive manner. The pA_2 values of TI-189 and TI-233 were 5.24 ± 0.21 and 6.23 ± 0.32 respectively.

2 Even at 10^{-4} M they had no inhibitory effect on the contractile response to noradrenaline (NA), histamine, prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$, arachidonic acid or potassium in rabbit aortic strips.

3 In dog basilar and coronary arterial strips and also in rat fundus, both agents inhibited the 5-HT response in a non-competitive manner.

4 At 10^{-5} M, TI-233 but not TI-189 antagonized effects of NA and KCl in the dog basilar and coronary arteries.

5 These arginine derivatives decreased the contractile responses induced by a prostaglandin endoperoxide analogue (U-44069) in rabbit aorta and in dog basilar and coronary arteries but there was no evidence for competitive antagonism.

6 These results indicate that the arginine derivatives are competitive antagonists selective for 5-HT receptors in rabbit aorta.

Introduction

The newly synthesized arginine derivatives, TI-189 and TI-233 (Okamoto, Hijikata, Kinjo, Kikumoto & Tamao, 1975; Okamoto, Hijikata, Ikezawa, Kinjo, Kikumoto, Tonomura & Tamao, 1976) were developed as thrombin inhibitors. Their chemical structures and anti-thrombin activities are shown in Figure 1. In addition, during the investigation of platelet aggregation inhibition, we found these arginine compounds had competitive inhibitory actions on 5-hydroxytryptamine (5-HT)-induced contractions of rabbit aortic strips. To our knowledge, these are the only substances structurally unrelated to 5-HT that are competitive inhibitors of 5-HT in vascular smooth muscle. Therefore the present experiments were undertaken to investigate their selectivity of action in several isolated preparations.

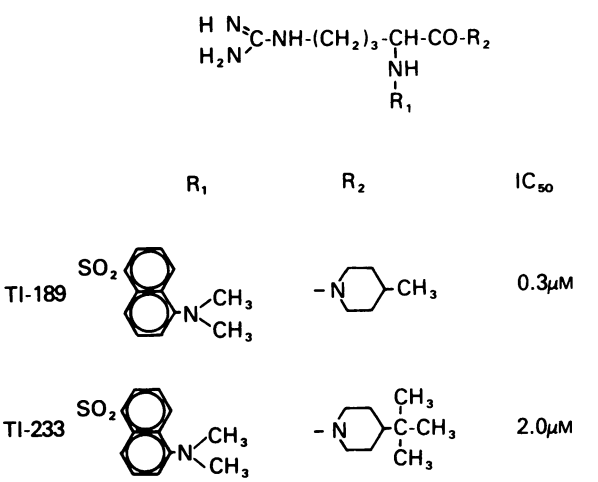

Figure 1 Chemical structure and antithrombin activity of TI-189: 4-methyl-1-[N_2-(5,6-dimethylaminonaphthalene-1-sulphonyl-L-arginyl]-piperidine and TI-233: 4-isopropyl-1-[N_2-(5,6-dimethylaminonaphthalene-1-sulphonyl-L-arginyl]-piperidine.

Methods

Rabbit aorta

Male New Zealand white rabbits weighing 1 to 2 kg were killed by a blow on the head and the chest was

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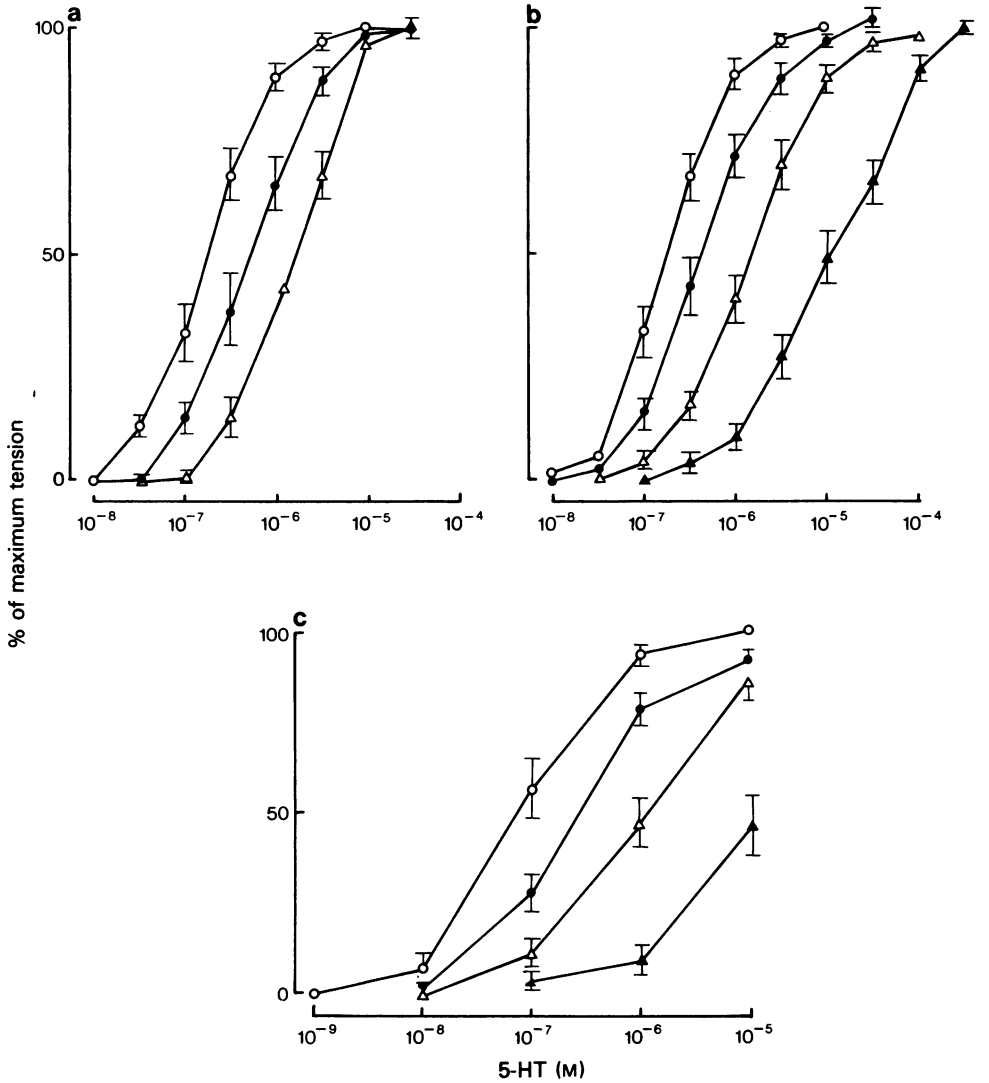

Figure 2 The effect of TI-189 (a), TI-133 (b) and methysergide (c) on the dose-response curves to 5-hydroxytryptamine (5-HT) in rabbit aortic strips: (a) (○) control; (●) TI-189 10^{-5} M; (△) TI-189 5×10^{-5} M. In (b), (○) control; (●) TI-233 10^{-6} M; (△) TI-233 3×10^{-6} M; (▲) TI-233 10^{-5} M. In (c), (○) control; (●) methysergide 10^{-8} M; (△) methysergide 10^{-7} M; (▲) methysergide 10^{-6} M. Each point and bar represents the mean and s.e. mean of 12 experiments.

opened to remove the thoracic aorta. After excess fat and connective tissue were removed, the aortae were cut into helical strips, about 5 mm in width and 40 mm in length, and mounted vertically in organ baths containing 20 ml of Krebs solution of the following composition (mM): NaCl 120.3, KCl 2.4, $CaCl_2$ 1.2, $MgSO_4.7H_2O$ 1.3, KH_2PO_4 1.2, $NaHCO_3$, 24.2 and glucose 5.5 at pH 7.4. The tissue bath solution was maintained at 37°C and bubbled with a 95% O_2 and 5% CO_2 gas mixture. Ligatures were placed around both ends of the muscle strips, one attaching the muscle to a glass holder and the other to a transducer adjusted to give an initial tension of 1.0 g. The preparations were allowed to equilibrate for 2 h in the medium before any drugs were added. Isometric tension changes were recorded through a force-displacement transducer (FT-0.3) connected to a seven channel Grass polygraph.

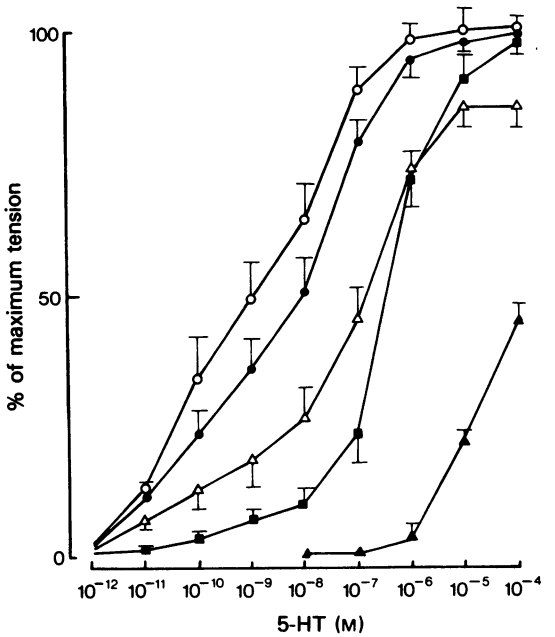

Figure 3 The effect of TI-189 and TI-233 on the dose-response curves to 5-hydroxytryptamine (5-HT) in dog basilar arteries: (○) control; (●) TI-189 10^{-6} M; (△) TI-189 10^{-5} M; (■) TI-233 10^{-6} M; (▲) TI-233 10^{-5} M. Each point and bar represents the mean and s.e. mean of 12 experiments.

Dog basilar and coronary arteries

Mongrel dogs of either sex, weighing 7.0 to 15.0 kg, were anaesthetized with pentobarbitone 35 mg/kg (i.v.) and exsanguinated from the common carotid arteries. The brain and heart were removed to dissect a basilar artery and a circumflex coronary artery. Each artery was cut into a helical strip (about 1.5 mm in width and 25 mm in length for the basilar artery, and 3 mm by 30 mm for the coronary artery) and set up at an initial tension of 1.5 g. Isometric contractions of these arteries to drugs were recorded as for rabbit aorta.

Rat fundus strip

Male Wistar rats, weighing 200 to 250 g, were killed with a blow on the head and the stomach was removed. The fundus portion of the stomach was dissected along the limiting ridge and cut into two pieces. Stomach fundus strips were prepared according to Perry (1970) and suspended at an initial tension of 1.0 g in an organ bath containing Krebs solution. The solution was aerated by a mixture of 95% O_2 and CO_2 and maintained at 37°C.

Full cumulative concentration-response curves were obtained by stepwise increases in concentration of the agonists: additions were made as soon as a steady state response was obtained from the preceding dose. The tissue was incubated with the arginine derivatives or methysergide for 10 min before addition of the agonist to the bath. EC_{50} values were

Table 1. Effect of TI-189 and TI-233 on the contractile responses to KCl and noradrenaline (NA) in the dog basilar and coronary arteries

	Treatment	*EC_{50} (M)*	*Maximum tension (%)*
		Basilar artery	
KCl	—	$17.6 \pm 2.5 \times 10^{-3}$	100
	TI-189 (10^{-5} M)	$18.5 \pm 2.3 \times 10^{-3}$	98.6 ± 2.3
	TI-233 (10^{-6} M)	$18.8 \pm 3.4 \times 10^{-3}$	97.4 ± 2.8
	TI-233 (10^{-5} M)	$29.3 \pm 3.7 \times 10^{-3}$*	50.0 ± 4.6*
NA	—	$7.5 \pm 0.7 \times 10^{-7}$	100
	TI-189 (10^{-5} M)	$7.2 \pm 0.4 \times 10^{-7}$	98.4 ± 2.4
	TI-233 (10^{-6} M)	$8.1 \pm 0.6 \times 10^{-7}$	98.0 ± 3.8
	TI-233 (10^{-5} M)	$1.6 \pm 0.4 \times 10^{-6}$*	57.6 ± 6.5*
		Coronary artery	
KCl	—	$15.8 \pm 2.5 \times 10^{-3}$	100
	TI-189 (10^{-4} M)	$17.6 \pm 1.6 \times 10^{-3}$	98.0 ± 2.4
	TI-233 (10^{-6} M)	$18.0 \pm 2.6 \times 10^{-3}$	96.0 ± 4.8
	TI-233 (10^{-5} M)	$26.3 \pm 1.3 \times 10^{-3}$*	48.4 ± 3.2*
NA	—	$3.8 \pm 9.7 \times 10^{-8}$	100
	TI-189 (10^{-5} M)	$4.4 \pm 0.5 \times 10^{-8}$	97.3 ± 3.6
	TI-233 (10^{-6} M)	$4.7 \pm 1.0 \times 10^{-8}$	96.4 ± 4.1
	TI-233 (10^{-5} M)	$9.8 \pm 0.3 \times 10^{-7}$*	56.2 ± 1.2*

Values of EC_{50} and maximum tension represent mean ± s.e. mean.
* Significant difference from control values (Student's t test, $P < 0.05$).

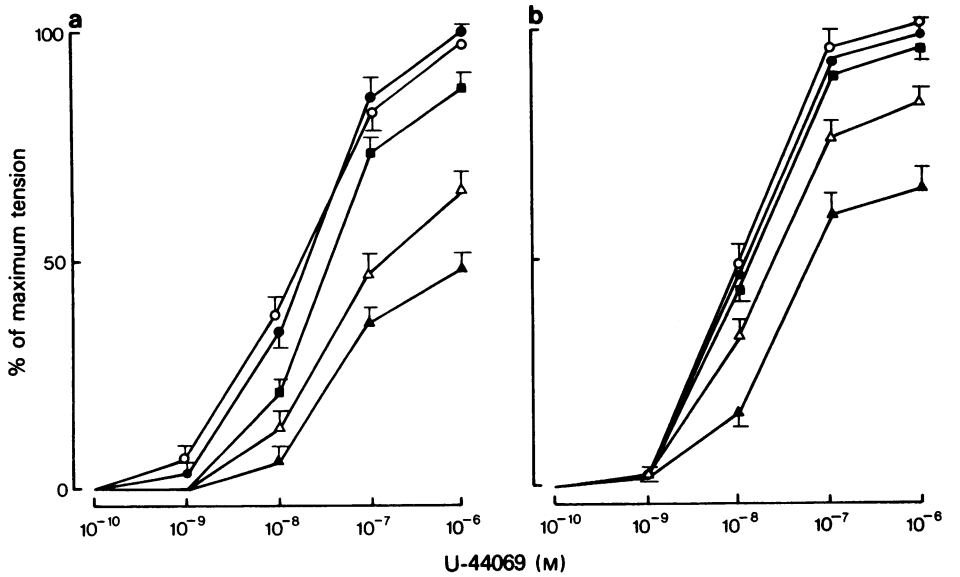

Figure 4 The effect of TI-189 and TI-233 on the dose-response curves to prostaglandin endoperoxide analogue, U-44069, in dog basilar (a) and coronary (b) arteries: (○) control; (●) TI-189 10^{-6} M; (△) TI-189 10^{-5} M; (■) TI-233 10^{-6} M; (▲) TI-233 10^{-5} M. Each point and bar represents the mean and s.e. mean of 7 experiments.

obtained from dose-response curves before and after antagonist treatment and each preparation was used only once. pA_2 values were calculated according to Van Rossum (1963) in rabbit aorta. Significant differences were indicated by a P value <0.05 in Student's t-test.

Drugs

Aqueous solutions of TI-189 and TI-233 (alcohol solubility high but water solubility low) were made by adding 1 N HCl dropwise to the aqueous suspension of the compounds to give a clear solution, which was then neutralized by alkali without formation of a precipitate. The following agents were used: methysergide maleate, noradrenaline bitartrate (Sigma Chem. Corp.), 5-hydroxytryptamine creatinine phosphate (Sandoz Pharmaceut. Corp), histamine dihydrochloride (Mann Res. Corp), PGE_2 (Ono Pharmaceut. Corp.), $PGE_{2\alpha}$ (Ono Pharmaceut. Corp.), arachidonic acid (Sigma Chem. Corp.) prostaglandin endoperoxide analogue ((15s)-hydroxy-9,11-(epoxymethano) prosta-5z, 13-E-dienoic acid, U-44069, Upjohn) and KCl (Sigma Chem. Corp.). Stock solutions of $PGF_{2\alpha}$, U-44069 and arachidonic acid were made up in absolute ethanol and further diluted with water. PGE_2 solution was diluted with 0.02% Na_2CO_3 solution and further diluted with water. The effect of solvents at various concentrations were tested but had no effect on the drug-induced contraction of the vascular smooth muscles.

Results

Rabbit aortic strips

Both TI-189 (10^{-6} M to 5×10^{-5} M) and TI-233 (10^{-6} M to 10^{-5} M) shifted the dose-response curve for 5-HT to the right in a parallel manner (Figure 2a, b) without altering the maximal response, which was 3.3 ± 0.3 g ($n = 12$). The pA_2 values were 5.24 ± 0.21 and 6.23 ± 0.32 for TI-189 and TI-233 respectively. However, no inhibitory effects were seen on NA, histamine, PGE_2, $PGF_{2\alpha}$, arachidonic acid or KCl even when a concentration of 10^{-4} M of each arginine derivative was used; neither were there any changes in basal tension with TI-189 or TI-233.

The sensitivity of rabbit aortae to the PG-endoperoxide analogue U-44069 varied markedly between different preparations and so full dose-response curves were not estimated. However, in 10 experiments where the interaction was studied, a response of 1.48 ± 0.23 g tension to U-44069 (1.5×10^{-9} to 3×10^{-9} M) was reduced to 0.31 ± 0.21 g and 0.12 ± 0.08 g in the presence of 10^{-5} M TI-189 and TI-233 respectively.

Methysergide (10^{-8} to 10^{-6} M) shifted the 5-HT dose-response curve to the right, although the basal tension was elevated by 0.35 ± 0.3 and 1.1 ± 0.17 g with 10^{-7} and 10^{-6} M methysergide respectively. Furthermore, the maximum response to 5-HT was

also reduced by these concentrations (by approximately 20% and 50% respectively) suggesting that the antagonism was not of a simple competitive type (Figure 2c).

Dog basilar artery

In this preparation, pretreatment with TI-189 and TI-233 (10^{-5} M and 10^{-6} M) shifted the dose-response curve for 5-HT to the right (Figure 3). At 10^{-5} M, TI-189 slightly decreased the maximum contractile response to 5-HT, whereas TI-233 greatly reduced it (Figure 3); furthermore, TI-233 but not TI-189 (10^{-5} M) inhibited the contractile response to KCl and NA (Table 1).

The treatment with either TI-189 or TI-233 at 10^{-5} M but not 10^{-6} M also significantly decreased the maximal contractile response to endoperoxide analogue U-44069 (10^{-10} M to 10^{-6} M) (Figure 4a). On both the dog basilar and coronary arteries (unlike rabbit aorta) the dose-response curves for U-44069 were reproducible, since the sensitivity of both tissues to the drug was consistent among different preparations.

Dog coronary arteries

At 10^{-7} M, neither TI-189 nor TI-233 altered the dose-response curve to 5-HT in the dog coronary arterial strip. However, higher concentrations (10^{-6} M and 10^{-5} M) shifted the response curve to the right and also decreased the maximum contractile response to 5-HT (Figure 5).

The contractile responses to both NA and KCl were reduced by 10^{-5} M TI-233 but unaffected by 10^{-5} M TI-189 (Table 1). Furthermore, at 10^{-5} M but not 10^{-6} M both TI-233 and TI-189 shifted the dose-response curve for U-44069 to the right with decrease in the maximum response (Figure 4b). However, the inhibitory action of the thrombin inhibitors on U-44069 was much greater in rabbit aortic strips than in the dog basilar and coronary arterial strips.

Rat stomach fundus

In rat stomach fundus strips 10^{-6} M TI-233 significantly shifted the dose-response curve for 5-HT to the right without reducing the maximum tension although the maximum tension was considerably reduced when the curve was further shifted to the right in the presence of 10^{-5} M (Figure 6). TI-189 did not affect the 5-HT response at a concentration of 10^{-5} M, but shifted the dose-response curve for 5-HT to the right and also reduced the maximum tension at higher concentrations of 3×10^{-5} M and 10^{-4} M (Figure 6).

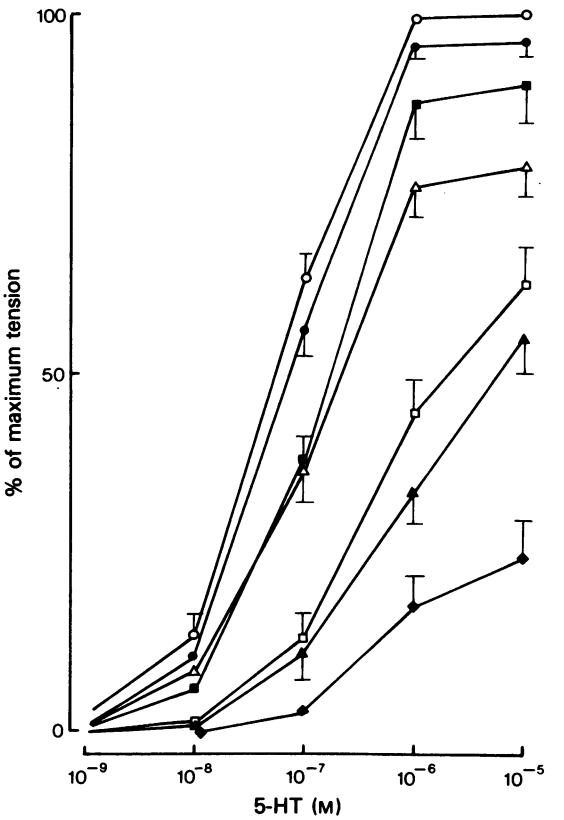

Figure 5 The effect of TI-189 and TI-233 on the dose-response curves to 5-hydroxytryptamine (5-HT) in the dog coronary arteries: (○) control; (●) TI-189 10^{-7} M; (△) TI-189 10^{-6} M; (▲) TI-189 10^{-5} M; (■) TI-233 10^{-7} M; (□) TI-233 10^{-6} M; (◆) TI-233 10^{-5} M. Each point and bar represents the mean and s.e. mean of 10 experiments.

Discussion

It is interesting to note that although the chemical structures of TI-189 and TI-233 are not related to that of 5-HT, both compounds had anti-5-HT action on the vascular smooth muscles. In rabbit aortic strips in particular, both compounds inhibited the 5-HT response in a competitive manner. However, in dog basilar and coronary arteries (and also the rat fundic strip) the inhibitory action was noncompetitive in type. On the basis of the pA_2 value for anti-5-HT action on the rabbit aorta, the potency of TI-233 was greater than that of TI-189 by about 10 fold. On the other hand, methysergide itself caused vascular contraction and was not a pure competitive antagonist on the aortic 5-HT receptor since it also reduced the amplitude of the maximal contraction. Furthermore,

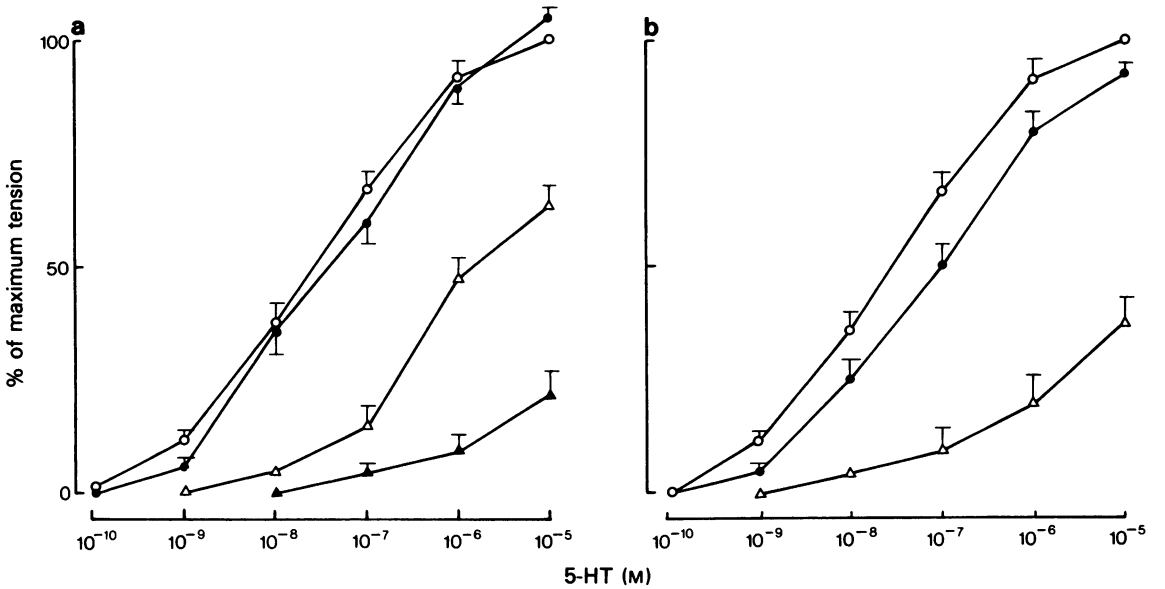

Figure 6 The effect of TI-189 (a) and TI-233 (b) on the dose-response curve to 5-hydroxytryptamine (5-HT) in the rat fundus: (a) (○) control; (●) TI-189 10^{-5} M; (△) TI-189 3×10^{-5} M; (▲) TI-189 10^{-4} M. In (b), (○) control; (●) TI-233 10^{-6} M; (△) TI-233 10^{-5} M. Each point and bar represents the mean and s.e. mean of 6 experiments.

TI-189 and TI-233 even at high concentrations did not have any inhibitory action on the contractile response to NA, histamine, PGE_2, $PGF_{2\alpha}$, arachidonic acid and KCl in the aortic strips. Therefore, in rabbit aortic strips, the inhibitory action of these compounds on the 5-HT receptor was quite selective.

In dog basilar and coronary arteries, TI-233 but not TI-189, at a high concentration (10^{-5} M) had a potent inhibitory action on the contractile response to NA and KCl. Thus, TI-233 is only a selective antagonist to 5-HT on rabbit aorta, but is a less selective antagonist in dog basilar and coronary arteries.

It has been demonstrated that the excitatory receptor for 5-HT in the dog femoral artery differs from that in the dog saphenous vein; it was further suggested that there are two types of excitatory receptors for 5-HT in dog vasculature (Apperley, Humphrey & Levy, 1977; Feniuk, Humphrey & Levy, 1977). For one type, methysergide and cyproheptadine are potent, competitive 5-HT antagonists and for the other type, methysergide is an agonist and cyproheptadine is a weak, non-competitive antagonist (Feniuk *et al.*, 1977). As shown in the present experiment also, the antagonistic action of both TI-189 and TI-233 on the 5-HT receptor in the rabbit aorta differs from that in the dog basilar and coronary arteries. Thus, the present results also support the possible existence of two different vascular receptors for 5-HT in blood vessels.

Both TI-189 and TI-233 had inhibitory actions on the PG-endoperoxide analogue (U-44069) in vascular smooth muscle though this inhibition was not of a competitive type. Recently, these arginine derivatives were also shown to inhibit the rabbit aortic contractile response to thromboxane A_2-like substances released during human platelet aggregation and to inhibit human platelet aggregation (our unpublished data).

In conclusion, therefore, these thrombin inhibitors appeared to be a type of competitive anti-5-HT agent on the rabbit aortic strip but to inhibit non-competitively the responses to 5-HT and U-44069 in other vascular smooth muscles. Coupled with their effects on blood platelets, these actions suggest that further study of these arginine derivatives might lead to the discovery of a useful agent for the treatment of cardiovascular diseases such as stroke, myocardial infarction and thrombosis.

Reprint requests to S.S. (Hawaii), please.

References

APPERLEY, E., HUMPHREY, P.P.A. & LEVY, G.P. (1977). Two types of excitatory receptor for 5-hydroxytryptamine in dog vasculature? *Br. J. Pharmac.*, **61**, 465P.

FENIUK, W., HUMPHREY, P.P.A. & LEVY, G.P. (1977). Further evidence for two types of excitatory receptor for 5-hydroxytryptamine in dog vasculature. *Br. J. Pharmac.*, **61**, 466P.

OKAMOTO, S., HIJIKATA, A., KINJO, K., KIKUMOTO, R. & TAMAO, Y. (1975). A novel series of synthetic thrombin-inhibitors having extremely potent and highly selective action. *Kobe J. Med. Sci.*, **21**, 43–51.

OKAMOTO, S., HIJIKATA, A., IKEZAWA, K., KINJO, R., KIKUMOTO, S., TONOMURA, S. & TAMAO, Y. (1976). A new series of synthetic thrombin-inhibitors (OM-Inhibitors) having extremely potent and selective action. *Thrombosis Res.*, **8** (supp. 11), 77–82.

PERRY, W.L.M. (1970) ed. In *Pharmacological Experiments on Isolated Preparations.* 2nd Ed. pp. 88–89. Edinburgh: Churchill Livingstone.

ROSSUM, J.M.VAN (1963). Cumulative dose-response curves 11. Technique for the making of dose-response curve in isolated organs and the evaluation of drug parameters. *Archs int. Pharmacodyn.*, **143**, 299–330.

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