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Tranilast, an anti-allergic drug, possesses antagonistic potency to angiotensin II

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Abstract

N-(3′,4′-dimethoxycinnamoyl) anthranilic acid (tranilast), an effective anti-allergic drug, has successfully prevented restenosis in patients who have undergone percutaneous transluminal coronary angioplasty. To elucidate the mechanism of tranilast, we investigated its antagonistic effect to angiotensin II, which plays a pivotal role in the proliferation of vascular smooth muscle cells, using angiotensin II-induced contractions in human gastroepiploic artery and rabbit aorta. The possible antagonistic effects of other anti-allergic agents such as 4-(*p*-chlorobenzyl)-2-(hexahydro-1-methyl-1H-azepin-4-yl)-1(2H)-phthalazinone hydrochloride (azelastine), 9-methyl-3-(1H-tetrazol-5-yl)-4H-pyrido[1,2-a]pyramidin-4-one potassium salt (pemirolast) and disodium cromoglycate were also compared. Tranilast dose-dependently inhibited the angiotensin II-induced contractions in human and rabbit arteries ($IC_{50} = 3.6 \times 10^{-5}$ M and $pD'_2 = 3.69$, respectively). Pemirolast showed a weak antagonistic effect to angiotensin II, but the effective concentration cannot be administered in clinical dosage. Tranilast and pemirolast had no effect on the concentration–contractile response curves for KCl and norepinephrine. Azelastine inhibited angiotensin II-, KCl- and norepinephrine-induced contractions non-specifically, while disodium cromoglycate did not affect these contractile responses. Tranilast but not azelastine showed synergistic action with 2-ethoxy-1-[[2′-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]-1H-benzimidazole-7-carboxylic acid (CV-11974) in antagonizing angiotensin II-induced contraction and the inhibitory pattern was similar to that of the non-peptide angiotensin II AT_1 receptor antagonist CV-11974. These findings indicate that only tranilast possesses the unique ability to antagonize angiotensin II in clinical dosage, which may contribute at least in part to prevention of restenosis after percutaneous transluminal coronary angioplasty.

Keywords: Tranilast; Azelastine; Pemirolast; Disodium cromoglycate; Angiotensin II receptor; Vessels, human

1. Introduction

N-(3′,4′-dimethoxycinnamoyl) anthranilic acid (tranilast) has been used widely for the treatment of allergic diseases such as bronchial asthma, allergic rhinitis, and atopic dermatitis, for which its inhibitory effect on the release of chemical mediators from mast cells is the key mechanism (Azuma et al., 1976; Komatsu et al., 1988). Tranilast also showed inhibitory effects on keloids and hypertrophic scars (Isaji et al., 1987; Suzawa et al., 1992). Recently, an additional action of tranilast in preventing restenosis after percutaneous transluminal coronary angioplasty (The TREAT Study Investigators, 1994) has been revealed, although its precise mechanism remains unknown.

Restenosis after percutaneous transluminal coronary angioplasty occurs as a result of the migration of vascular smooth muscle cells from the media to the intima within the arterial wall, where the cells proliferate and overproduce extracellular matrix (Liu et al., 1989; Macleod et al., 1994). Although the mechanism of restenosis after percutaneous transluminal coronary angioplasty has not been generally understood, some studies revealed the possible involvement of angiotensin II in the development of intimal hyperplasia or hypertrophy by interacting with vascular smooth muscle cells in an autocrine and/or paracrine manner. In cultured cells, angiotensin II induces an increase in RNA, protein synthesis and cell size with little or no increase in DNA synthesis and cell number, and hypertrophy may result (Geisterfer et al., 1988). On the other hand, angiotensin II can also induce a significant increase in DNA synthesis as well as in growth rates and cell number, resulting in hyperplasia (Rosendorff, 1996). In the

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rat balloon-injury model, not only angiotensin converting enzyme inhibitors but also angiotensin II AT_1 receptor antagonists can prevent and/or attenuate neointima formation (Powell et al., 1989; Osterrieder et al., 1991). In addition, platelet-derived growth factor (PDGF) (Ferns et al., 1991) and basic FGF (Lindner and Reidy, 1991) have also been reported to be implicated in the development of intimal hyperplasia or hypertrophy after arterial injury in rats. However, several studies of cultured vascular smooth muscle cells showed that angiotensin II can directly stimulate the synthesis of PDGF (Okuda et al., 1996) and basic FGF (Itoh et al., 1993). These findings indicate that angiotensin II is a direct mediator in regulating vascular smooth muscle cells proliferation to induce restenosis.

In the present study, the potential of tranilast to antagonize angiotensin II was investigated in the isolated human and rabbit arteries. The possible angiotensin II antagonistic effects of other anti-allergic agents, 4-(*p*-chlorobenzyl)-2-(hexahydro-1-methyl-1H-azepin-4-yl)-1(2H)-phthalazinone hydrochloride (azelastine) (Fischer and Schmutzler, 1981; Gould et al., 1988), 9-methyl-3-(1H-tetrazol-5-yl)-4H-pyrido[1,2-a]pyramidin-4-one potassium salt (pemirolast) (Yanagihara et al., 1988, 1989) and disodium cromoglycate (Cox, 1967), all of which, like tranilast, are characterized by inhibition of the release of chemical mediators from mast cells, were also explored.

2. Materials and methods

2.1. Drugs and tissue preparation

Four anti-allergic drugs (tranilast, pemirolast, azelastine, and disodium cromoglycate) were generously provided by the Kissei Pharmaceutical (Nagano, Japan). 2-Ethoxy-1-[[2′-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]-1H-benzimidazole-7-carboxylic acid (CV-11974) was a gift from Nippon Roussel (Tokyo, Japan). Angiotensin II was purchased from Peptide Institute, (Osaka, Japan).

The gastroepiploic arteries were isolated from resected stomachs of 12 patients who underwent gastrectomy for gastric carcinomas in our hospital. The patients ranged in age from 32 to 82 years. Descending thoracic aortas were removed from male Japanese White rabbits (Japan SLC, Shizuoka) weighing 2.5–3.3 kg, under sodium pentobarbital anesthesia (60 mg/kg, i.v.). The experimental procedures for humans and animals were conducted respectively in accordance with the guidelines of Osaka Medical College for medical experiments edited by the ethics community that included outside members.

2.2. Effects of anti-allergic drugs on contractions induced by various vasoconstrictors in human and rabbit arteries

Human gastroepiploic arteries and rabbit aortas were cut into helical strips 2.0-mm wide and 20-mm long and 3.0-mm wide and 20-mm long, respectively. The strips were mounted in a 10 ml tissue bath containing Tyrode's solution composed of 137 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 1.1 mM $MgCl_2$, 0.42 mM NaH_2PO_4, 12 mM $NaHCO_3$ and 5.7 mM glucose. Tyrode's solution was kept at 37°C, pH 7.4, and bubbled continuously with 5% CO_2 in oxygen. The initial resting tensions were set at 1.5 g for the human arterial strips and 2.0 g for the rabbit aortic strips. Isometric tension signals were detected with a force-displacement transducer (TB-611T, Nihon Kohden, Tokyo, Japan), amplified and displayed on a chart recorder (U-228, Pantos, Kyoto, Japan). To avoid possible interference with responses to angiotensin II by endogenous prostanoids of which biosynthesis and release are affected by angiotensin II, 1 μM indomethacin was added to the medium throughout the experiments. After an equilibration period of 90–120 min, the strips were stimulated by adding 30 mM KCl, and the response of each strip served as the reference for agonist-induced contraction of the corresponding strip. The contractile response for angiotensin II (3×10^{-8} M) on human gastroepiploic strips was obtained first. The cumulative concentration–response curves for angiotensin II (1×10^{-10}–3×10^{-7} M) on the rabbit aortic strips were also obtained first. After the maximum contractile response was reached, the strips were rinsed four times over a 1-h period and were allowed to relax to baseline tension. The third or fourth response was regarded as the control response for the respective strips, because the responses to angiotensin II were stabilized with sufficient reproducibility after the second or third repeated doses. After the control responses to angiotensin II were constructed in each strip, the strips were rinsed four times within a 30-min period, and then various concentrations of anti-allergic drugs were added to the tissue bath and allowed a 30-min contact prior to the addition of angiotensin II. On the other hand, the cumulative concentration–contractile response curves for KCl (10–60 mM) and norepinephrine (1×10^{-8}–3×10^{-5} M) were also constructed in the rabbit aorta. After cumulative concentration–contractile response curves for KCl and norepinephrine could be stably reproduced, the strips were rinsed four times within a 30-min period. Thereafter, various concentrations of anti-allergic drugs were added to the tissue bath and allowed a 30-min contact prior to the addition of these agonists. To measure the antagonistic potency of these agonists, the pD'_2 and pA_2 values were determined according to the methods described by Van Rossum (1963).

2.3. Synergistic experiments

After reproducible contractile responses for angiotensin II (3×10^{-8} M) were produced in the rabbit aorta, a single dose of tranilast (1×10^{-4} M), azelastine (1.85×10^{-4} M) or CV-11974 (1×10^{-10} M) was added to the respective tissue bath and a 30-min contact was allowed prior to the addition of angiotensin II. In another tissue bath, the

strips were synthetically treated with CV-11974 and tranilast or azelastine. Double CV-11974 (2×10^{-10} M) was also used for treatment in other strips to determine its additional action. After a 30-min co-incubation period, the strips were rechallenged with the same concentration of angiotensin II. The inhibitory potency to angiotensin II for these drugs concentrations is less than the IC_{50}.

2.4. Statistical analysis

All numerical data were expressed as the mean ± S.E.M. Differences were considered significant when the P values were less than 0.05 with Duncan's test.

3. Results

In the rabbit aortas, tranilast at 3×10^{-5}, 1×10^{-4}, and 3×10^{-4} M caused a flattening of the concentration–contractile response curves for angiotensin II and reduced the maximal contractile responses to angiotensin II by 16.0 ± 6.1%, 31.3 ± 6.3% and 61.3 ± 5.7%, respectively (Fig. 1A). The calculated pD'_2 of tranilast was 3.69 ± 0.09. Azelastine also flattened the concentration–contractile response curves for angiotensin II, and the maximal contractile responses to angiotensin II were reduced by 9.2 ± 6.9% and 94.3 ± 2.7% at 1×10^{-4} and 3×10^{-4} M, respectively (Fig. 1B). A pD'_2 value of 3.77 ± 0.02 for azelastine was calculated. Pemirolast, even at 3×10^{-4} M, flattened the concentration–contractile response curve for angiotensin II and the maximal contractile response to angiotensin II was reduced by 19.2 ± 6.7% (Fig. 1C). A pD'_2 value of 2.91 ± 0.18 for pemirolast was calculated. As shown in Fig. 1D, disodium cromoglycate up to 3×10^{-4} M did not affect the concentration–contractile response curve for angiotensin II in the rabbit aortas.

As shown in Fig. 2, in the human gastroepiploic artery, 3×10^{-8} M angiotensin II-induced contractile responses were suppressed by tranilast in a dose-dependent manner and were almost completely abolished by adding 3×10^{-4} M tranilast. Azelastine, like tranilast, also suppressed the angiotensin II-induced contractile responses dose-dependently. Pemirolast dose-dependently inhibited the contractile responses to angiotensin II, but the antagonistic potency of pemirolast was less than that of tranilast and azelastine ($p < 0.01$). Disodium cromoglycate at any concentration did not affect the contractile responses to angiotensin II. IC_{50} values obtained with tranilast, azelastine and pemirolast were 3.6 ± 0.9 ($\times 10^{-5}$) M, 5.2 ± 0.6 ($\times 10^{-5}$) M and 2.5 ± 0.7 ($\times 10^{-4}$) M, respectively (Fig. 2).

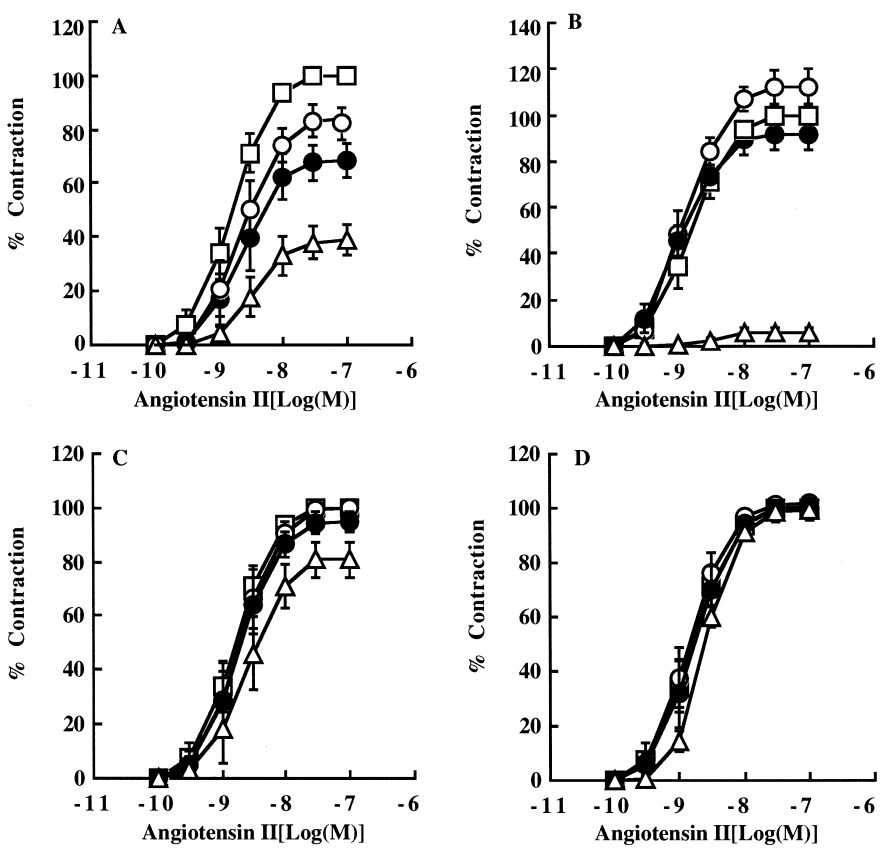

Fig. 1. Effects of tranilast (A), azelastine (B), pemirolast (C) and disodium cromoglycate (D) on the concentration–contractile response curves for angiotensin II in rabbit isolated aortic strips. Results are expressed as a percentage of the maximal response to angiotensin II before addition of the drugs and are the mean ± S.E.M. Control (□), 3×10^{-5} M (○), 1×10^{-4} M (●) and 3×10^{-4} M (△) ($n = 4$).

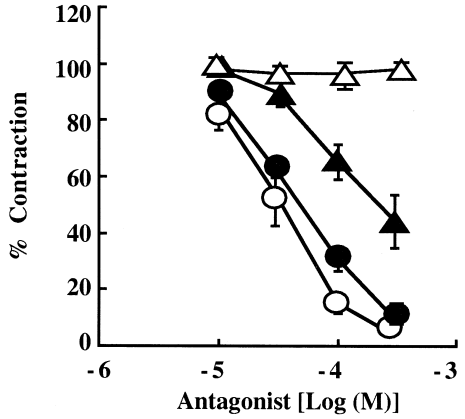

Fig. 2. Effects of tranilast (○), azelastine (●), pemirolast (▲) and disodium cromoglycate (△) on the contractile responses evoked by 3×10^{-8} M angiotensin II in human gastroepiploic arteries. Results are expressed as a percentage of the response to angiotensin II before addition of the drugs and are the mean ± S.E.M. ($n=6$).

Tranilast, pemirolast and disodium cromoglycate between concentrations of 3×10^{-5} and 3×10^{-4} M did not affect the concentration–contractile response curves for KCl (10–60 mM) and norepinephrine (1×10^{-8}–3×10^{-5} M) in the rabbit aorta (Figs. 3 and 4A, C, D). On the contrary, azelastine dose-dependently inhibited both KCl- and norepinephrine-induced concentration–contractile response curves (Fig. 3B and 4B). Azelastine flattened the KCl-induced concentration–contractile response and reduced the maximal contractile response in the rabbit aorta and a pD'_2 value of 3.80 ± 0.12 (Fig. 3B) was calculated. On the other hand, azelastine at 3×10^{-5} M shifted the concentration–contractile response curves for norepinephrine to the right without altering the maximal contractile response in the rabbit aorta, and the calculated pA_2 value was 7.9 ± 0.1 (Fig. 4B). However, when the concentration of azelastine was raised from 3×10^{-5} M to 1×10^{-4}–3×10^{-4} M, inhibition was shifted from competitive to noncompetitive. The pD'_2 value obtained with azelastine was 3.68 ± 0.05 (Fig. 4B).

As shown in Fig. 5, single doses of tranilast (1×10^{-4} M), CV-11974 (1×10^{-10} M) and azelastine (1.85×10^{-4} M) suppressed the maximal contractile responses to angiotensin II (3×10^{-8} M) by 45.7 ± 4.6%, 38.0 ± 6.5% and 49.9 ± 4.1%, respectively. When the strips were co-incubated with tranilast and CV-11974 or double CV-11974, the angiotensin II-induced contractions were additionally inhibited compared with the contractions treated with a single dose of tranilast or CV-11974 (78.4 ± 4.5% for the group treated with tranilast and CV-11974 and 73.6 ± 12.0% for the group treated with double CV-11974). Con-

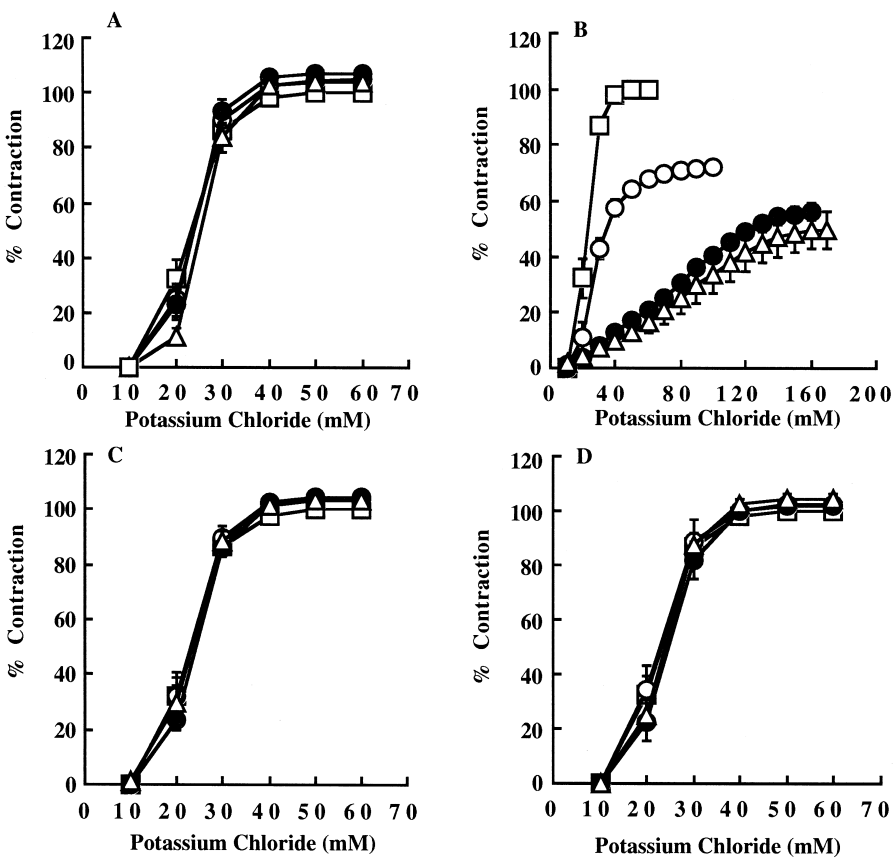

Fig. 3. Effects of tranilast (A), azelastine (B), pemirolast (C) and disodium cromoglycate (D) on the concentration–contractile response curves for potassium chloride (KCl) in rabbit isolated aortic strips. Results are expressed as a percentage of the maximal response to potassium chloride before addition of the drugs and are the mean ± S.E.M. Control (□), 3×10^{-5} M (○), 1×10^{-4} M (●) and 3×10^{-4} M (△) ($n=4$).

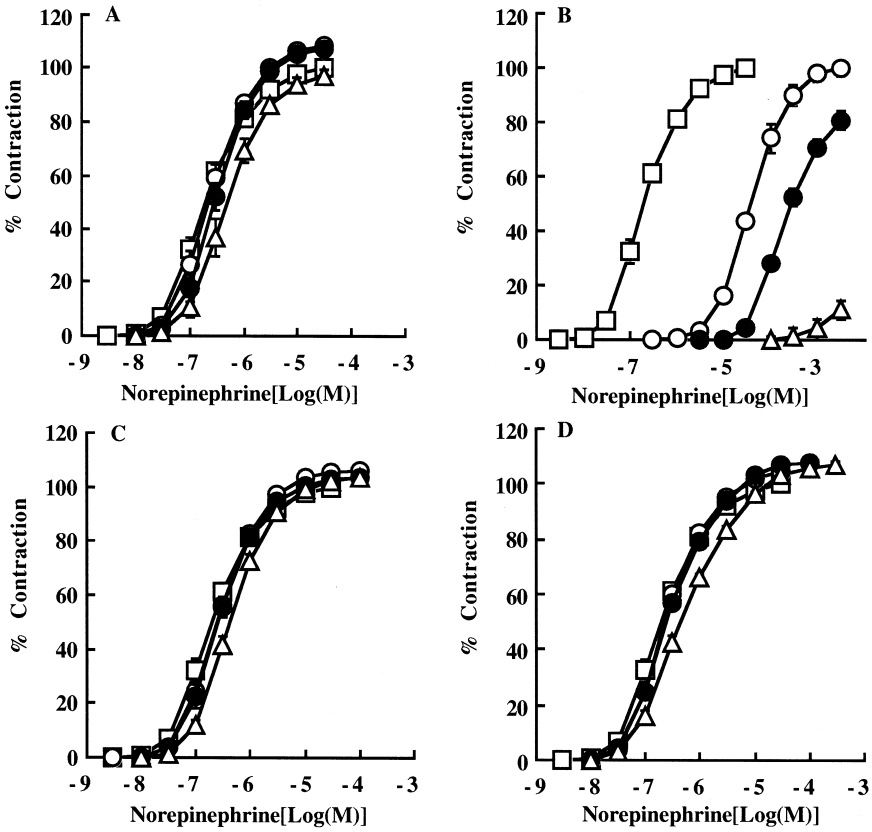

Fig. 4. Effects of tranilast (A), azelastine (B), pemirolast (C) and disodium cromoglycate (D) on the concentration–contractile response curves for norepinephrine in rabbit isolated aortic strips. Results are expressed as a percentage of the maximal response to norepinephrine before addition of the drugs and are the mean ± S.E.M. Control (□), 3×10^{-5} M (○), 1×10^{-4} M (●) and 3×10^{-4} M (△) ($n = 4$).

versely, when the strips were co-incubated with azelastine and CV-11974, no further inhibition was observed on the angiotensin II-induced contractions compared with the contractions treated with a single dose of azelastine (57.3 ± 4.1% vs. 49.9 ± 4.1%).

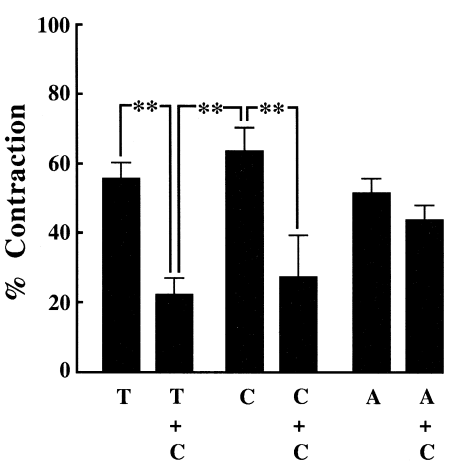

Fig. 5. Effects of 1×10^{-4} M tranilast (T), 1.85×10^{-4} M azelastine (A) and 1×10^{-10} M CV-11974 (C) alone or combined with CV-11974 on the contractile responses evoked by 3×10^{-8} M angiotensin II in rabbit arteries. Results are expressed as a percentage of the response to angiotensin II before addition of the drugs and are the mean ± S.E.M. ($n = 4$). $** p < 0.01$ vs. the value compared.

4. Discussion

In human arteries, tranilast inhibited angiotensin II-induced contraction in a dose-dependent manner. In rabbit aorta, tranilast was shown to be an insurmountable antagonist of angiotensin II-induced contraction. On the other hand, tranilast did not affect the concentration–contractile response curves induced by KCl and norepinephrine in the rabbit aorta. Tranilast also had no effect on the contractions induced by endothelin-1 (data not shown). The pharmacological behavior of tranilast was similar to that of specific angiotensin II AT1 receptor antagonists such as CV-11974 and 2-Butyl-4-(methylthio)-1-[[2′-[[[(propylamino)carbonyl] amino]sulfonyl](1,1′-biphenyl)-4-yl]methyl]-1H-imidazole-5-carboxylate (HR720) (Shibouta et al., 1993; Jin et al., 1997), although the antagonistic potency of tranilast to angiotensin II was somewhat smaller than that of CV-11974 and HR720. In synergistic experiments, when tranilast but not azelastine was synthetically treated with CV-11974, the angiotensin II-induced contractions were additionally inhibited compared with the con-

tractions treated with a single dose of tranilast and the inhibitory pattern was similar to the non-peptide angiotensin II AT_1 receptor antagonist CV-11974. These results suggest that tranilast may interact with the same angiotensin II AT_1 receptor levels. Conversely, azelastine, which showed non-specific inhibition to the contractile responses induced by all agonists in the present studies, did not exhibit synergistic action with CV-11974 in antagonizing angiotensin II-induced contractions. This result also shows that the inhibitory effects of azelastine on the contractions induced by the agonists were mainly due to its non-specific inhibition. These findings, taken together, indicating the antagonistic effects of tranilast to angiotensin II are specific to angiotensin II AT_1 receptors, which are responsible for the known biological actions of angiotensin II (Timmermans et al., 1993). The plasma concentration could reach approximately 1×10^{-4} M in humans with clinical dosage of tranilast (600 mg/day) administered orally (Tanaka et al., 1994). Under a tranilast concentration such as that applied in the present study, 85% of angiotensin II-induced contraction appeared to be suppressed in the human arteries. Therefore, it is clear that tranilast could exhibit an antagonistic effect to angiotensin II in clinical administration.

Pemirolast at high concentration also showed an antagonistic effect to angiotensin II specifically, but this effective concentration cannot be practically obtained in plasma by oral administration. In the human arteries, pemirolast at over 1×10^{-4} M showed a weak antagonistic effect to angiotensin II. However, the plasma concentration only reached approximately 3×10^{-6} M after a standard clinical dosage of pemirolast (10 mg/day) administered orally (Hasegawa et al., 1994). Apparently, the concentration at which the antagonistic effect to angiotensin II appears on contractile systems and the actual attainable level in plasma are separated by a factor of approximately 30.

On the other hand, azelastine inhibited the contractile responses induced by angiotensin II, KCl and norepinephrine in the human and rabbit arteries, although its inhibitory properties were somewhat different. In the angiotensin II-induced contractions, azelastine depressed the force of contraction only in the highest concentration (in rabbit aorta), and in the KCl-induced contractions, it produced dose-dependent depression but not complete depression, whereas the norepinephrine contraction curve is clearly shifted to the right for the low concentration. Such nonspecific inhibition to contractile systems may be explained by the fact that azelastine, like a genuine Ca^{2+} antagonist, inhibits voltage-gated Ca^{2+} inward, agonist-induced Ca^{2+} release, and Ca^{2+} sensitization (Lee et al., 1990; Masuo et al., 1992). Although there are no clinical and animal experimental studies on azelastine for the evaluation of possible preventive efficacy in post-percutaneous transluminal coronary angioplasty, a previous report demonstrated that the Ca^{2+}-channel antagonist verapamil had no effect on neointima formation in balloon-injured models (Powell et al., 1991). In contrast, disodium cromoglycate did not affect angiotensin II-, KCl- or norepinephrine-induced contractions in the human or rabbit arteries.

The present study showed that of the four anti-allergic drugs examined, only tranilast could be used at an effective concentration to antagonize angiotensin II in clinical dosage. These results strongly suggest that the mechanism of preventing restenosis after percutaneous transluminal coronary angioplasty by tranilast may be due to its antagonistic effect on angiotensin II.

Recently, it has been reported that there are non-peptide angiotensin II receptor antagonists such as losartan (Timmermans et al., 1991), TCV-116 (Shibouta et al., 1993) and HR720 (Jin et al., 1997), all of which are imidazole derivatives. In this study, pemirolast, like tranilast, had a specific antagonistic effect on angiotensin II. However, both drugs are not imidazole derivatives, and the chemical structure of tranilast is also very different from that of pemirolast. Therefore, it is unclear how these drugs specifically inhibit angiotensin II action, and further study is needed to elucidate the mechanisms involved.

We conclude that among the anti-allergic drugs examined, only tranilast specifically possesses an antagonistic effect on angiotensin II in a concentration useful in the clinic, and this effect may prevent the development of restenosis after percutaneous transluminal coronary angioplasty.

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