British Journal of Pharmacology (1996) 117, 967-973

KW-4679-induced inhibition of tachykininergic contraction in the guinea-pig bronchi by prejunctional inhibition of peripheral sensory nerves

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1 Sensory mechanisms play an important role in the vagal regulation of tracheobronchial smooth muscle tone. We examined the effect of KW-4679, an anti-allergic drug, on guinea-pig tachykinin-mediated contractile responses induced by electrical field stimulation (EFS) in guinea-pig bronchial muscles.

2 EFS (8 Hz, 0.5 ms, 15 V, for 15 s) evoked biphasic contractile responses in the guinea-pig isolated main bronchus in the presence of 5 μM indomethacin. The contractions consisted of a fast phase of an atropine-sensitive transient contraction and a slow phase of a sustained contraction which was inhibited by a combination of the tachykinin NK_1 receptor antagonist, (±)-CP-96,345 (1 μM) and the NK_2 receptor antagonist, SR 48969 (0.1 μM).

3 KW-4679 preferentially inhibited the slow phase in a concentration-dependent manner by 43.2±7.7% at 10 μM, whereas the drug had no effect on the fast phase at concentrations up to 10 μM. KW-4679, at a concentration of 100 μM, inhibited not only the slow phase by 49.2±11.4%, but also the fast phase by 36.8±8.4%.

4 KW-4679 (10 μM and 100 μM) did not affect the substance P-induced or neurokinin A-induced contraction. Against the acetylcholine-induced contractile responses, 100 μM KW-4679 had a marked effect producing a 10.2 fold shift to the right in the curve.

5 The inhibitory effect of KW-4679 (10 μM) on the slow phase contraction was not influenced by treatment with naloxone (100 nM), propranolol (1 μM), thioperamide (1 μM), saclofen (50 μM), yohimbine (1 μM), methiothepin (1 μM) or methysergide (1 μM).

6 The inhibitory effect of KW-4679 (10 μM) on the slow phase contraction was not influenced by treatment with intermediate or large conductance Ca^{2+}-activated K^+ channel blockers (charybdotoxin (10 nM) or iberiotoxin (10 nM)), but suppressed by treatment with small conductance Ca^{2+}-activated K^+ channel blockers, apamin (500 nM) or scyllatoxin (300 nM). Apamin or scyllatoxin *per se* did not influence the slow phase contractions.

7 The results suggest that KW-4679 preferentially inhibits the release of tachykinins from the bronchial sensory nerves through activation of small conductance Ca^{2+}-activated K^+ channels.

Keywords: KW-4679; electrical field stimulation; guinea-pig isolated bronchi; apamin; scyllatoxin; tachykinin; Ca^{2+}-activated K^+ channels

Introduction

Tachykinins induce bronchoconstriction (Lundberg *et al.*, 1987) and neurogenic inflammation (Szolcsányi, 1988). Barnes (1986) proposed that stimulation of the sensory nerves in airways may lead to tachykinin release via an axon reflex. Laitinen *et al.* (1985) reported that tachykinins may contribute partly to the inflammatory response in airways of asthmatic patients. Moreover, Bertrand *et al.* (1993) demonstrated that antigen-induced bronchoconstriction in guinea-pigs involved neurogenic inflammation. Taken together, modulation of the actions of tachykinins in airways should lead to the attenuation of the airway neurogenic inflammation and bronchoconstriction.

Grundström *et al.* (1981) reported that electrical field stimulation (EFS) elicits a biphasic contraction in guinea-pig bronchus. The fast phase of contraction is mediated by acetylcholine (ACh) released from parasympathetic nerves, and the slow phase by tachykinins released from unmyelinated sensory nerves. It has been shown that the tachykinin-mediated response is inhibited presynaptically by the activation of several receptors such as the μ-opioid receptor (Ray *et al.*, 1991b), the α_2-adrenoceptor (Matran *et al.*, 1989), the γ-aminobutyric acid $(GABA)_B$ receptor (Belvisi *et al.*, 1989; Ray *et al.*, 1991a), the histamine H_3 receptor (Ichinose *et al.*, 1989) the neuropeptide Y (NPY) receptor (Matran *et al.*, 1989), the 5-hydroxytryptamine (5-HT) receptor (Broad *et al.*, 1993) and the adenosine A_2 receptor (Morimoto *et al.*, 1993). It is reported that charybdotoxin (ChTX) prevented the inhibitory modulation by several agonists in the airway sensory nerves (Lou & Lundberg, 1993; Stretton *et al.*, 1992). Hence it is suggested that activation of ChTX-sensitive intermediate and large conductance Ca^{2+}-activated K^+ channels (IK_{Ca} channels and BK_{Ca} channels, respectively) may be involved in the inhibitory modulation of the sensory nerves.

KW-4679 ((Z)-11-[(3-dimethylamino)propylidene]-6,11-dihydrodibenz[*b,e*]oxepin-2-acetic acid monohydrochloride) is an anti-allergic drug (Ohshima *et al.*, 1992). KW-4679 has relatively potent and selective anti-histamine H_1 receptor antagonist activity. The pK_B value against histamine is 7.7 in

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guinea-pig ileum (Sasaki *et al.*, 1995). KW-4679 also has inhibitory activity against release of histamine from rat peritoneal mast cells at 10 μM. It was found that the compound inhibits antigen-induced airway hyperresponsiveness and late asthmatic response in actively sensitized guinea-pigs (Ohmori *et al.*, 1993). The precise mechanisms of the actions of KW-4679 remain unclear.

The aim of this study was to explore the effect of KW-4679 on tachykinin-mediated contractions in the airway smooth muscles. We have investigated the effect of KW-4679 on the EFS-induced contraction of the main bronchi of guinea-pigs, and demonstrated the inhibition of the tachykinin-mediated contraction by KW-4679. Thus, we attempted to determine the site of action and mechanism of KW-4679 using selective receptor antagonists and Ca^{2+}-activated K^+ channel blockers.

Methods

Tissue preparation

Male Hartley guinea-pigs (SLC, Japan), weighing 300–700 g, were killed with carbon dioxide gas, and the trachea-bronchial tree was excised. Four main bronchi rings, 1.5–2 mm in width each, were dissected. The tissues were individually mounted on two 'L' shaped stainless steel holders in organ baths filled with Krebs Henseleit solution maintained at 37°C and oxygentated with 95% O_2: 5% CO_2. The Krebs Henseleit solution had the following composition (mM): NaCl 119, KCl 4.7, $CaCl_2 2H_2O$ 2.5, $MgSO_4$ 1.2, $KH_2PO_4 H_2O$ 1.2, $NaHCO_3$ 25 and glucose 11.7. All the experiments were performed in the presence of indomethacin (5 μM) to prevent the formation of contractile prostaglandins. Contractile responses were recorded with an isometric force transducer (TB-611T; Nihon Kohden, Japan) and a recorder (LR4220; Yokogawa, Japan). A resting tension of 0.3 g, which has been found to be optimal for measuring changes in tension, was applied to the preparations. The tissues were then allowed to equilibrate for 1 h, during which they were washed every 20 min.

Electrical field stimulation-induced guinea-pig bronchial contractions

Electrical field stimulation (EFS) was applied with a stimulator (SEN3301; Nihon Kohden) via two platinum electrode rods inserted inside and outside of the ring preparation. EFS comprised rectangular pulses of 8 Hz frequency, 0.5 ms duration and 15 V for 15 s. EFS induced reproducible bronchial contractions when the preparation was stimulated up to three times.

Effect of KW-4679, receptor antagonists and K^+ channel blockers

After two stable EFS-induced bronchial responses had been obtained, KW-4679 was added at least 10 min before one test response to EFS, in a non-cumulative manner with only one concentration of KW-4679 added per tissue. The incubation times of tetrodotoxin, atropine, capsaicin, (±)-CP-96345 and SR 48968 were 60 min, 10 min, 30 min, 20 min and 20 min respectively. The incubation times of K^+ channel blockers exclusive of ChTX were 10 min. The incubation time of ChTX was 20 min. The effects of selective receptor antagonists and K^+ channel blockers were investigated both on bronchial responses alone and on the possible inhibition of these responses produced by KW-4679 (10 and 100 μM), using four bronchial ring preparations derived from one animal. Vehicle, KW-4679 alone, KW-4679 plus drug and drug alone were applied to each preparation at least 10 min before EFS-induced bronchial responses.

Effects on exogenously applied agonist-induced contractile response

The effects of KW-4679 on the contractile responses induced by cumulatively applied acetylcholine (10 nM–0.3 mM), substance P (1 nM–1 μM) or neurokinin A (0.1 nM–300 nM) were examined in the guinea-pig bronchial preparation. A concentration-response relationship for each agonist was obtained in the absence or presence of KW-4679 (10, 100 μM). KW-4679 was applied to the preparation 10 min before the cumulative addition of each agonist. The pD_2 values were derived from the log concentration-effect curves and are defined as the negative log of the agonist concentration that caused 50% of maximal effect.

Statistical analysis

Values are indicated as the mean ± s.e. The effect of the test compound was expressed as percentage or percentage inhibition of the amplitude of the contraction prior to the addition of the compound. Statistical differences were analyzed by Student's *t* test for paired data and for unpaired data or by Tukey test when appropriate. *P* values less than 0.05 were considered statistically significant.

Drugs

KW-4679, (±)-CP-96345 (dihydrochloride salt of a racemic mixture containing both (2S, 3S-*cis*– and (2R, 3R-*cis*–2(diphenylmethyl)-*N*-((2-methoxyphenyl)methyl)-1-azabicyclo[2.2.2]octan-3-amine) and SR 48968 ((S)-*N*-methyl-*N*-[4-(4-acethylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl) butyl] benzamide) were synthesized in our laboratories. Other drugs and chemicals were obtained from following sources; substance P (SP), neurokinin A (NKA), acetylcholine hydrochloride (ACh), apamin, tetrodotoxin, pyrilamine maleate, (±)-propranolol hydrochloride and yohimbine hydrochloride were purchased from Sigma Co. (St Louis, MO, U.S.A). Charybdotoxin, iberiotoxin and scyllatoxin were purchased from Peptide Institute Inc. (Osaka, Japan). Thioperamide maleate and 2-hydroxy saclofen were purchased from Research Biochemicals Inc. (Natick, MA, U.S.A). Chlorpheniramine maleate was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Atropine sulphate, indomethacin and capsaicin were purchased from Wako Pure Chemical Ind. Ltd. (Osaka, Japan). Peptides were dissolved in distilled water and stored below −40°C. Capsaicin and indomethacin were dissolved in ethanol. (±)-CP-96345 and SR 48968 were dissolved in dimethylsulphoxide. Other drugs were dissolved in distilled water.

Results

Inhibitory effect of KW-4679 on EFS-induced bronchial contraction

EFS induced reproducible biphasic contraction at least three times in bronchial smooth muscles (Figure 1a). Biphasic contractile responses were markedly suppressed by treatment with 3 μM tetrodotoxin (Figure 1b). The fast phase and the slow phase of the response were selectively inhibited by atropine (0.3 μM) and a combination of the NK_1 receptor antagonist (±)-CP-96345 (1 μM) and the NK_2 receptor antagonist SR 48968 (0.1 μM), respectively (Figure 1c and 1d). After incubation with capsaicin (30 μM) for 30 min, the slow phase of contractile response was abolished (Figure 1e). These results were consistent with previous findings (Kamikawa & Shimo, 1993a, b) and indicated that the fast and slow phase were mediated by ACh and tachykinins (SP and NKA), respectively.

Figure 2c illustrates the effects of KW-4679 on the EFS-

induced response. KW-4679 at 10 μM attenuated the slow phase of EFS-induced contraction ($P<0.05$) without affecting the fast phase (Figure 2a). At a concentration of 100 μM, KW-4679 inhibited not only the slow phase of contraction ($P<0.05$), but also the fast phase ($P<0.01$) (Figure 2b). In the presence of atropine (0.3 μM), KW-4679 at concentrations of 10 and 100 μM inhibited the EFS-induced contraction significantly by 31.5 ± 5.1% ($n=7$, $P<0.05$) and 51.5 ± 3.0% ($n=6$, $P<0.05$), respectively.

Effects of KW-4679 on exogenously applied acetylcholine-, substance P- and neurokinin A-induced bronchial contraction

KW-4679 had no effect on the basal tone by itself (data not shown). Figure 3 shows the effect of KW-4679 on the guinea-pig bronchial contraction. Against the SP-induced contractile responses, KW-4679 (10 or 100 μM) had no inhibitory effect.

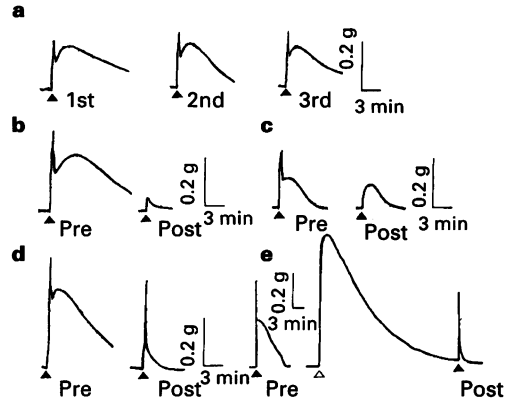

Figure 1 Typical tracings of EFS (0.5 ms, 8 Hz, 15 v for 15 s)-induced guinea-pig bronchial contractions in the presence of 5 μM indomethacin: (▲) EFS. (a) Repeated EFS-induced bronchial contractions. (b) Effect of tetorodotoxin treatment: The guinea-pig bronchus was incubated with 3 μM tetrodotoxin for 60 min. (c) Effect of atropine treatment: The guinea-pig bronchus was incubated with 0.3 μM of atropine for 10 min. (d) Effect of tachykinin antagonists: The guinea-pig bronchus was incubated with 1 μM of the NK_1-antagonist, (±)-CP-96345 and 0.1 μM of the NK_2-antagonist, SR 48968 for 20 min. (e) Effect of capsaicin treatment: The guinea-pig bronchus was challenged with 30 μM capsaicin for 30 min as indicated by (△).

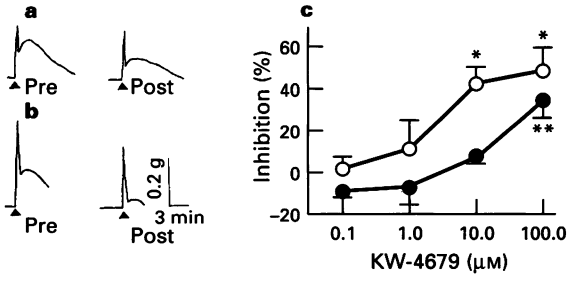

Figure 2 Effect of KW-4679 on EFS (0.5 ms, 8 Hz, 15 v for 15 s)-induced guinea-pig bronchial contractions for 20 min. Typical tracings of EFS-induced bronchial contractions in the presence of 10 μM (a) or 100 μM (b) KW-4679; (▲) indicates EFS. (c) Concentration-dependent inhibition of the EFS-induced bronchial contractions by KW-4679; (●) fast phase contractions (○) slow phase contractions. Values are shown as the mean ±s.e. ($n=4-11$). Significant differences from vehicle-treated group are indicated by: $^{*}P<0.05$, $^{**}P<0.01$ (Student's t test).

The pD_2 values for SP were 7.01 ± 0.12, 7.07 ± 0.07 and 6.81 ± 0.15 in the absence and presence of KW-4679 (10 and 100 μM), respectively ($n=6$, $P>0.05$). Against the NKA-induced contractile responses, KW-4679 tended to induce a slight rightward shift of the concentration-response curve at 100 μM. The pD_2 values for NKA were 7.66 ± 0.04, 7.55 ± 0.05 and 7.34 ± 0.16 in the absence and presence of 10 or 100 μM KW-4679, respectively ($n=6$, $P>0.05$). Against the ACh- induced contractile responses, KW-4679 has a minor inhibitory effect at 10 μM and had a marked effect producing

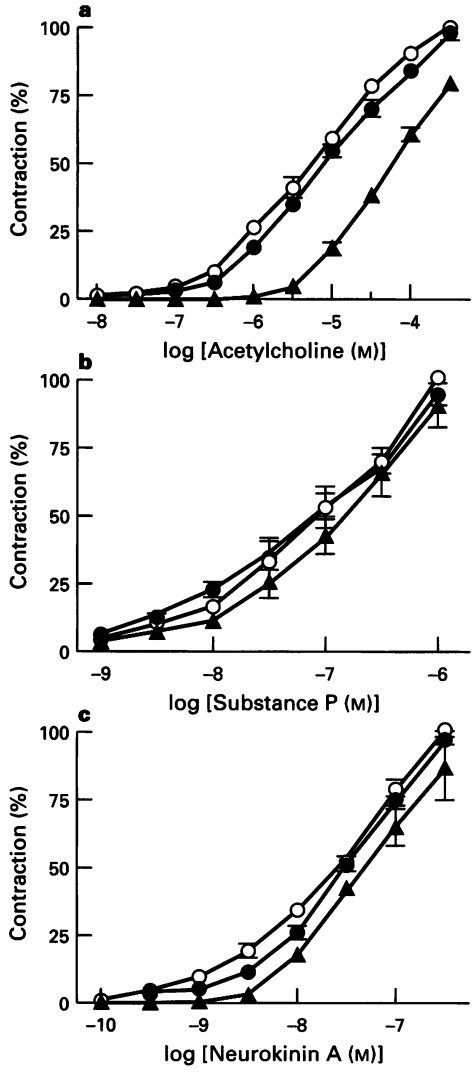

Figure 3 Effects of KW-4679 on exogenously applied acetylcholine (ACh)-induced, substance P (SP)-induced and neurokinin A (NKA)-induced guinea-pig bronchial contractions. The bronchi were contracted in the presence as control (○), 10 μM KW-4679 (●) or 100 μM KW-4679 (▲) for 10 min. The bronchi were contracted with ACh (a), SP (b) or NKA (c). The contractions are expressed as the maximum contractions with the highest concentration of agonists in the presence of vehicle. Values are shown as the mean ±s.e. Significant difference of pD_2 values between control and KW-4679-treated groups was analyzed by Tukey's test. The pD_2 values for ACh were 5.29 ± 0.02, 5.07 ± 0.05 and 4.28 ± 0.06 in the absence of and presence of 10 μM and 100 μM of KW-4679, respectively ($n=4$). There were significant differences between the control value and the value after treatment with 10 μM KW-4679 ($P<0.05$) and 100 μM KW-4679 ($P<0.01$). The pD_2 values for SP were 7.01 ± 0.12, 7.07 ± 0.07 and 6.81 ± 0.15 in the absence of and presence of 10 μM and 100 μM KW-4679, respectively, showing no significant difference between the values ($n=6$). The pD_2 values for NKA were 7.66 ± 0.04, 7.55 ± 0.05 and 7.34 ± 0.16 in the absence and presence of 10 μM or 100 μM KW-4679, respectively, showing no significant difference between the values ($n=6$).

a 10.2 fold shift to the right in the curve at 100 μM. The pD_2 values for ACh were 5.29 ± 0.02 and 4.28 ± 0.06 in the absence and presence of 100 μM of KW-4679 ($n=4$, $P<0.01$).

Influence of receptor antagonists on the inhibitory effect of KW-4679

Table 1 shows the effects of various receptor antagonists such as naxolone (0.1 μM), propranolol (1 μM), thioperamide (1 μM), saclofen (50 μM), yohimbine (1 μM) methiothepin (1 μM) and methysergide (1 μM) on the inhibitory effect of KW-4679 on the slow phase of EFS-induced contractile responses. None of the compounds *per se* affected the contractile response induced by EFS nor affected the inhibitory action of KW-4679.

Effects of histamine H_1 antagonists on EFS-induced bronchial contraction

Histamine H_1 antagonists, pyrilamine (0.1 – 10 μM) and chlorpheniramine (0.1 – 10 μM) had no influence on the EFS-induced bronchial contractions ($n=6$, $P>0.05$). Pyrilamine and chlorpheniramine, at a concentration of 10 μM, reduced the slow phase of the EFS-induced bronchial contractions by 18.4 ± 6.2% and by 11.5 ± 2.8%, respectively.

Influence of K^+ channel blockers on the inhibitory effect of KW-4679

ChTX (10 nM) did not change the basal tone and EFS-induced contractile responses. As reported by Stretton *et al.* (1992), ChTX, at a concentration of 10 nM, reversed the inhibitory effect of clonidine (100 nM) on the fast phase of and the slow phase of EFS-induced contraction (data not shown). ChTX (10 nM) failed to attenuate the inhibitory effect of KW-4679 (10 μM) (Figure 4a); the inhibition rates of KW-4679 in the absence and presence of ChTX were 61.3 ± 4.8% and 46.1 ± 4.0%, respectively ($n=7$, $P>0.05$). At a concentration of 30 nM, ChTX *per se* elicited bronchial contractile responses and augmented the fast phase and the slow phase of the EFS-induced contractile responses by 15.4 ± 6.5% and 77.5 ± 28.4%, respectively ($n=4$). Similarly, IbTX (10 nM), a BK_{Ca} channel blocker, failed to attenuate the inhibitory effect of KW-4679 (Figure 4b).

Apamin (500 nM), a SK_{Ca} channel blocker, which did not influence the bronchial basal tone and EFS-induced contraction, almost completely counteracted the inhibitory effect of KW-4679 (10 μM) against the EFS-induced slow phase of contraction, producing inhibition of 46.8 ± 10.8% and 2.7 ± 6.0% in the absence and presence of apamin, respectively ($n=6$, $P<0.01$) (Figure 5a). Furthermore, apamin partly attenuated the inhibitory effect of 100 μM KW-4679 on the fast phase and slow phase of the EFS-induced response (Figure 5b). KW-4679 (100 μM) inhibited the fast phase in the absence and presence of apamin by 48.5 ± 6.6% and 22.5 ± 5.9%, respectively ($n=4$, $P<0.01$). KW-4679 (100 μM) inhibited the slow phase in the absence and presence of apamin by 83.4 ± 4.6% and 31.0 ± 8.4%, respectively ($n=4$, $P<0.01$). Apamin, at a concentration of 500 nM, did not influence the SP-induced and the NKA-induced bronchial contractile responses (data not shown).

To confirm the participation of SK_{Ca} channels in the in-

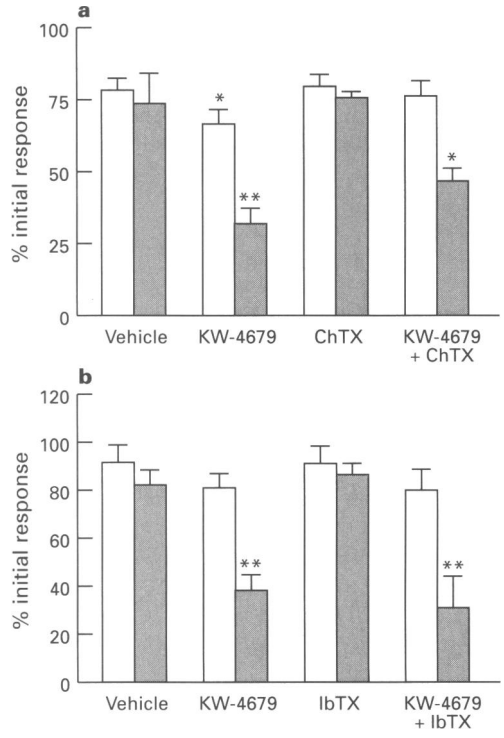

Figure 4 Effects of Ca^{2+}-activated potassium channel blockers on the inhibitory action of KW-4679 on EFS-induced bronchial contractions. Four bronchial rings isolated from the same guinea-pig were used. Each preparation was incubated with vehicle, 10 mM KW-4679, potassium channel blocker or a combination of both drugs. Charybdotoxin (ChTX, 10 nM) (a) or iberiotoxin (IbTX, 10 nM) (b) were incubated with preparations for 20 or 10 min, respectively. KW-4679 was incubated with preparations for 10 min. Open columns indicated the fast phase contractions induced by EFS and shaded columns indicate the slow phase contractions. Values shown are mean ± s.e. ($n=7$ or 5) ($^*P<0.05$, $^{**}P<0.01$ *vs.* vehicle treated group: $P>0.05$ between KW-4679-treated group and KW-4679-plus K^+ channel blockers-treated group, Tukey's test).

Table 1 Effects of receptor-antagonists on the inhibitory effect of KW-4679 on the slow phase of contractions induced by electrical field stimulation in the guinea-pig main bronchus

Receptor	*Drug*	*Conc.* (μM)	*Inhibition (%)* *KW-4679 alone*	*KW-4679 + drug*	n
μ-Opioid	Naloxone	0.1	41.1 ± 3.9**	36.7 ± 6.0*	5
β-Adrenoceptor	Propranolol	1.0	23.7 ± 8.8*	19.4 ± 5.8	8
H_3-histamine	Thioperamide	1.0	43.6 ± 17.1	32.6 ± 12.6	4
$GABA_B$	Saclofen	50	48.6 ± 9.6	37.1 ± 4.1	5
α_2-Adrenoceptor	Yohimbine	1.0	42.8 ± 8.2*	48.4 ± 9.6**	4
5-HT	Methiothepin	1.0	40.1 ± 4.7**	34.8 ± 4.7**	8
	Methysergide	1.0	47.3 ± 3.8**	38.3 ± 7.7*	5

Values are means ± s.e. n = number of guinea-pigs per group. The concentration of KW-4679 was 10 μM. $^*P<0.05$, $^{**}P<0.01$; significantly different compared to the vehicle-treated group (Tukey test). There is no significant difference between the group treated with KW-4679 alone and the group treated with a combination of KW-4679 and the respective receptor antagonist (Tukey test).

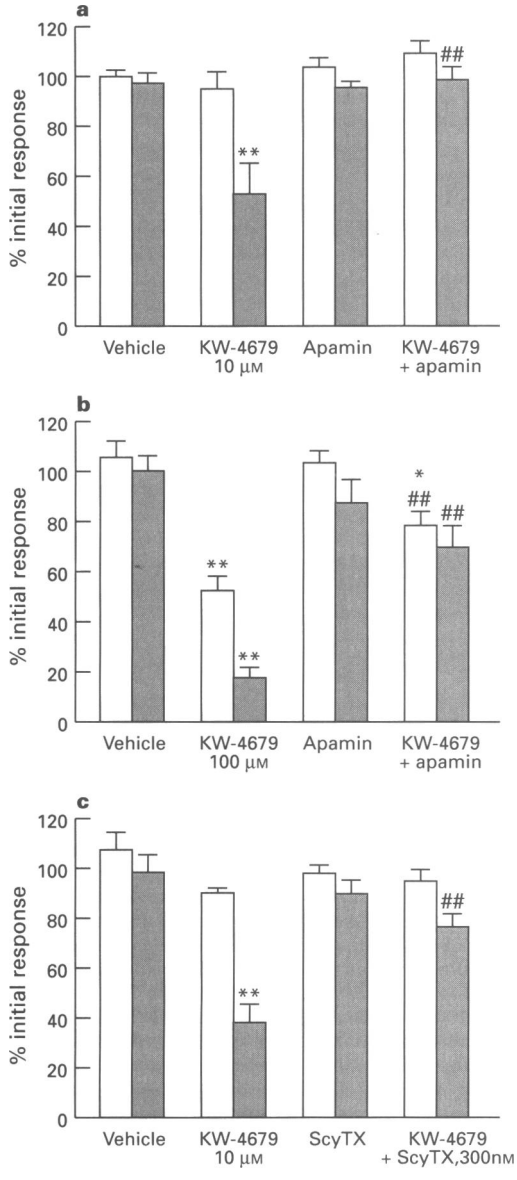

Figure 5 Reversal effects of apamin (500 nM) (a and b) and scyllatoxin(ScyTX,300n*M*) (c) on the inhibitory action of KW-4679 on the late phase of EFS-induced bronchial contractions. The concentrations of KW-4679 used are 10 μM (a and c) and 100 μM (b). Each preparation was incubated with or without drugs for 10 min. Open columns indicate the fast phase contractions and shaded columns indicate the slow phase contractions. Values show the mean $\pm$s.e. ($n=4-6$). Statistical analysis was by Tukey's test ($^{*}P<0.05$, $^{**}P<0.01$ *vs.* vehicle-treated group, $^{\#\#}P<0.01$ *vs.* KW-4679 alone-treated group).

hibitory effect of KW-4679 we used another SK_{Ca} channel blocker, scyllatoxin. Like apamin, scyllatoxin (300 nM), which did not influence the basal tone and EFS-induced contraction, attenuated the inhibitory effect of KW-4679 (Figure 5c). KW-4679 (10 μM) inhibited slow phase of the EFS-induced bronchial contraction in the absence and presence of scyllatoxin (300 nM) by 62.0 $\pm$ 7.7% and 23.6 $\pm$ 5.0%, respectively ($n=6$, $P<0.01$).

Discussion

It was reported that EFS-induced contractile responses of the guinea-pig bronchus involve the neurotransmitter release and the activation of cholinoceptors and tachykinin NK_1 and NK_2 receptors (Kamikawa & Shimo, 1993a, b). In this study, it was confirmed that the fast phase of the EFS-induced contraction is mediated by released acetylcholine and activation of muscarinic receptors, and the slow phase is mediated by released tachykinins (probably substance P and neurokinin A) and activation of both NK_1 and NK_2 receptors. In the present study, the fast phase and slow phase should be interpreted as the cholinergic contraction and as the tachykininergic contraction, respectively.

KW-4679 (1–100 μM) attenuated the EFS-induced tachykininergic bronchial contraction. KW-4679, at 10 μM, reduced preferentially the tachykininergic contraction without influencing the cholinergic contraction. There is a possibility that the bronchial contraction might be inhibited postjunctionally. However, KW-4679 (10 μM) did not affect the SP-induced and NKA-induced contractions. These results indicate that KW-4679's inhibitory effect on the tachykininergic contraction was mediated by prejunctional action on the sensory nerves. On the other hand, the reduction in the cholinergic contraction by KW-4679 (100 μM) may be attributable to the postjunctional action of KW-4679.

It is unlikely that antihistamine effect of KW-4679 contributes to the prejunctional action in the main bronchi, since the antihistamine compounds, chlorpheniramine and pyrilamine did not influence the tachykininergic contraction.

Recently, Verleden *et al.* (1994) showed that ketoifen inhibits the noncholinergic contraction through the activation of 5-HT receptors and that the inhibition is reversed by the 5-HT receptor antagonists, methiothepin or methysergide. To determine if any receptor activation participates in the effect of KW-4679, we used the following antagonists: μ-opioid receptor antagonist, naloxone; the α_2-adrenoceptor antagonist, yohimbine; the $GABA_B$ antagonist, saclofen; the histamine H_3 antagonist, thioperamide; the β-adrenoceptor antagonist, propranolol and the 5-HT receptor antagonists, methiothepin and methysergide. None of these significantly attenuated the inhibitory effect of KW-4679. At present, it is possible that KW-4679 stimulates receptors other than those explored in this study. Nevertheless the data suggest the differences between the inhibitory mechanism of action of ketotifen and KW-4679.

It was reported that the activation of ChTX-sensitive K^+ channels may be involved in the inhibitory modulation of peripheral nerves (Stretton *et al.*, 1992; Lou & Lundberg, 1993), but the inhibitory effect of KW-4679 was not influenced by the presence of 10 nM ChTX or IbTX, a BK_{Ca} channel blocker (Galvez *et al.*, 1990). Since 30 nM ChTX and 100 nM IbTX induced contractions of the bronchial smooth muscle and augmented the EFS-induced contraction, we could not investigate the effect of ChTX at 30 nM and the effect of IbTX at 100 nM. As reported by Stretton *et al.* (1992), ChTX, at a concentration of 10 nM, reversed the inhibitory action of clonidine (100 nM) on the fast phase and the slow phase of EFS-induced contraction (data not shown). Thus, the concentration of ChTX is sufficient to block the ChTX-sensitive K channels. The presence of another apamin-sensitive small conductance Ca^{2+}-activated K^+ (SK_{Ca}) channel in nerves has also been reported (Blatz & Magleby, 1986). Apamin (500 nM), a SK_{Ca} channel blocker (Kawai & Watanbe, 1986), did not alter the basal tone. In addition, apamin did not influence the EFS-induced, the SP-induced or the NKA-induced bronchial contractile responses. Unexpectedly, apamin abolished the inhibitory effect of 10 μM KW-4679 on the tachykininergic contraction and it attenuated the inhibitory effect of 100 μM KW-4679 on cholinergic and tachykininergic responses. The reason why apamin did not entirely abolish the inhibitory effect of KW-4679 on the cholinergic contraction is probably associated with the postjunctional action of KW-4679. Stretton *et al.* (1992) reported that apamin failed to reverse the inhibitory effect of clonidine on nonadrenergic and noncholinergic (NANC) contractile responses. This indicates that not all inhibitory effects of the NANC contractile responses are reversed by apamin. Therefore, it is considered that the effect of apamin has a se-

lectivity against the inhibitory effect of KW-4679 on the NANC contractile response. In order to ascertain that the inhibitory effect of KW-4679 involves SK_{Ca} channel activation, we used another SK_{Ca} channel blocker, scyllatoxin (Abia *et al.*, 1986; Auguste *et al.*, 1990). Scyllatoxin like apamin, attenuated the inhibitory effect of KW-4679 on the tachykininergic contraction. These results indicate that SK_{Ca} channel activation may be involved in the inhibitory action of KW-4679.

NANC-induced relaxation was reported in the EFS-induced guinea-pig bronchial contraction (Undem *et al.*, 1990) and, it was also reported that NANC-induced relaxation in the guinea-pig bronchial preparation is mediated by IbTX-sensitive or ChTX-sensitive K^+ channels and not by apamin-sensitive K^+ channels (Ellis & Conanan, 1994). In our study, the inhibitory effect of KW-4679 on the slow phase is influenced by apamin and not by ChTX or IbTX, so the possibility that KW-4679 may enhance or elicit the NANC-relaxation is excluded. We intend to investigate the influence of KW-4679 against inhibitory NANC regulation in airway smooth muscles in a future study.

In conclusion, the present study indicates that the anti-allergic drug KW-4679, presynaptically inhibited the tachykininergic contraction induced by EFS in the guinea-pig bronchus. The inhibitory effect of KW-4679 was mediated by SK_{Ca} channels activation. This is the first investigation to indicate that SK_{Ca} channels can modulate neurotransmitter release from sensory nerves in airways. To clarify whether SK_{Ca} channels are opened by EFS in the presence of KW-4679, further work, e.g., electophysiological studies, are needed. Since intramural tachykininergic nerves are known to play a pro-inflammatory role in the airways, the inhibition by KW-4679 of tachykinin release in airways seems to be beneficial for the treatment of airway inflammation and the symptons of bronchial asthma.

The authors wish to acknowledge Professor Y. Kamikawa for the advise and technical support.

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(Received May 15, 1995
Revised October 19, 1995
Accepted November 3, 1995)