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Inhibitory effects of sympathomimetic drugs on cholinergically mediated contractions of guinea-pig isolated tracheal muscle

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The experiments examine the actions of sympathomimetic drugs on the responses evoked by electrical field stimulation or by acetylcholine in guinea-pig tracheal strip chains. Electrical field stimulation evoked contractions which were cholinergically mediated, in the presence of guanethidine (10 μM) and indomethacin (2 μM). All the sympathomimetic drugs tested caused a concentration-dependent reduction in the height of these contractions. Inhibitory effects of isoprenaline and terbutaline were largely prevented by propranolol (2 μM) alone, whereas those of clonidine, oxymetazoline, lidamidine and WHR1370 were prevented by yohimbine alone (2 μM). Treatments with both propranolol and yohimbine were required to prevent the inhibitory effects of noradrenaline, adrenaline and dopamine. Contractions evoked by exogenous acetylcholine (0·1–3 μM) were also inhibited by all catecholamines and terbutaline, but not by clonidine, oxymetazoline, lidamidine and WHR1370. The inhibitory effects were antagonized by propranolol (2 μM) alone. The results suggest that in guinea-pig isolated tracheal muscle, sympathomimetic drugs can inhibit cholinergic neurotransmission not only by postjunctional β_2-adrenoceptors but also by prejunctional α_2-adrenoceptors.

It has long been recognized that mammalian airway smooth muscle is controlled by sympathetic adrenergic nerves causing relaxation and parasympathetic cholinergic nerves causing contraction. With the discovery of predominantly non-adrenergic inhibitory nerves in this tissue (Kamikawa & Shimo 1976b; Richardson & Béland 1976; Taylor et al 1984). The physiological role of adrenergic nerves has been re-examined. Recent evidence indicates that noradrenaline functions as a prejunctional inhibitor of cholinergic neurotransmission via α_2-adrenoceptors and adrenaline causes relaxation of airway smooth muscle via β_2-adrenoceptors (Vermeire & Vanhoutte 1979; Bryan et al 1981; Grundström et al 1981; Kamikawa 1982). Although many β_2-adrenoceptor stimulants such as terbutaline, have been developed to treat bronchial asthma, refractoriness or subsensitivity has been induced by prolonged medication (Nelson et al 1977; Shelhamer et al 1983). On the other hand, α_2-adrenoceptor stimulants, such as clonidine or oxymetazoline have been used in essential hypertension or nasal congestion, but have not been tested on bronchial asthma.

The present experiments were made to compare the inhibitory potency of various sympathomimetic drugs including α_2-stimulants on cholinergically mediated contractions of guinea-pig tracheal muscle with or without β-adrenoceptor blockade.

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MATERIALS AND METHODS

Male guinea-pigs (300 to 500 g) were stunned, the trachea excised and the tracheal strip chains were prepared (Kamikawa & Shimo 1976a,b). Briefly, four pieces of tracheal transverse strips, 2–3 mm wide, were connected in alignment with threads and immersed in a 10 ml organ bath filled with modified Krebs bicarbonate solution of the following composition (mM); NaCl 120, KCl 4·7, $CaCl_2$ 2·5, $MgCl_2$ 1·2, $NaHCO_3$ 25, KH_2PO_4 1·2, disodium edetate 0·03, ascorbic acid 0·12 and glucose 5·56 (pH 7·4). The Krebs solution always contained 20 μM choline chloride and was bubbled with 5% carbon dioxide in oxygen, and maintained at 37 °C.

The preparation was suspended under an initial tension of 0·5 g and 60 min was allowed to elapse before experiments were started. The tracheal response was isometrically recorded by means of a force-displacement transducer (Nihon Kohden SB-1T-H) and a Nihon Kohden polygraph recorder (RJG-4004). Electrical field stimulation was with rectangular pulses of 8 Hz frequency, 0·5 ms duration and supramaximal voltage, through bipolar platinum electrodes which were 10 mm apart and connected to a Nihon Kohden stimulator (SEN-1101). The total number of stimulating pulses was kept constant at 40. For the eliminations of sympathetic components and endogenous prostaglandin biosynthesis in responses to field stimulation, the Krebs solution contained 10 μM guanethi-

dine and 2 μM indomethacin. When the strip was electrically stimulated every 100 s, stable twitch-like contractions were obtained, the height of which was nearly equivalent to that of contractions evoked by exogenously supplied acetylcholine (ACh, 0·3–1 μM). The elicited twitch contractions seemed to be mediated by the stimulation of intramural cholinergic nerves in tracheal muscle, since they were completely prevented by tetrodotoxin (0·3 μM) and atropine (1 μM) (Kamikawa 1982). The effects of sympathomimetic drugs on the twitch contractions were measured as the percentage changes of the original twitch height obtained just before the drug was applied to the bath.

Drugs used were noradrenaline bitartrate, adrenaline bitartrate, isoprenaline bitartrate, yohimbine hydrochloride, propranolol hydrochloride (Sigma), tetrodotoxin, indomethacin (Sankyo), guanethidine sulphate (Ciba-Geigy), dopamine hydrochloride, atropine sulphate (Wako), ephedrine hydrochloride (Dainippon), acetylcholine chloride (Daiichi), lidamidine hydrochloride, 1-n-butoxy-3-(2,6-dimethyl phenoxycarbamoyl)guanidine hydrochloride (WHR1370, William H. Rorer), clonidine hydrochloride (Nippon Boehringer Ingelheim), terbutaline sulphate (Fujisawa), and oxymetazoline hydrochloride (Chugai). To prepare the drug solutions, catecholamines were dissolved in and diluted with 0·9% w/v NaCl solution (saline) containing 120 μM ascorbic acid; indomethacin was dissolved in distilled water containing equimolar concentrations of Na_2CO_3 and diluted with saline; all other drugs were dissolved in and diluted with saline. The molar concentrations of drugs refer to the final bath concentrations.

RESULTS

Inhibitory effects of sympathomimetic drugs on cholinergically mediated contractions of normal tracheal muscle

All the sympathomimetic drugs tested inhibited the twitch contractions of guinea-pig tracheal muscle evoked by electrical field stimulation. The inhibitory responses were concentration-dependent and reversible by washing. However, their inhibitory potencies were variable and isoprenaline was the most potent (Fig. 1). The relative potencies of adrenaline, noradrenaline, dopamine and terbutaline compared with that of isoprenaline (= 1) were 0·3, 0·1, 0·01 and 0·02, respectively, on the basis of each nM concentration required to inhibit the twitch by 50% (Table 1). Selective α_2-stimulants, clonidine, oxymetazoline, lidamidine and WHR1370, also inhibited the twitch contractions, but were less potent. As summarized in Table 1, maximum twitch inhibitions by catecholamines and terbutaline reached about 90–100%, while those by α_2-stimulants were less than 70% even at the highest concentrations examined. Ephedrine, an orally active bronchodilator,

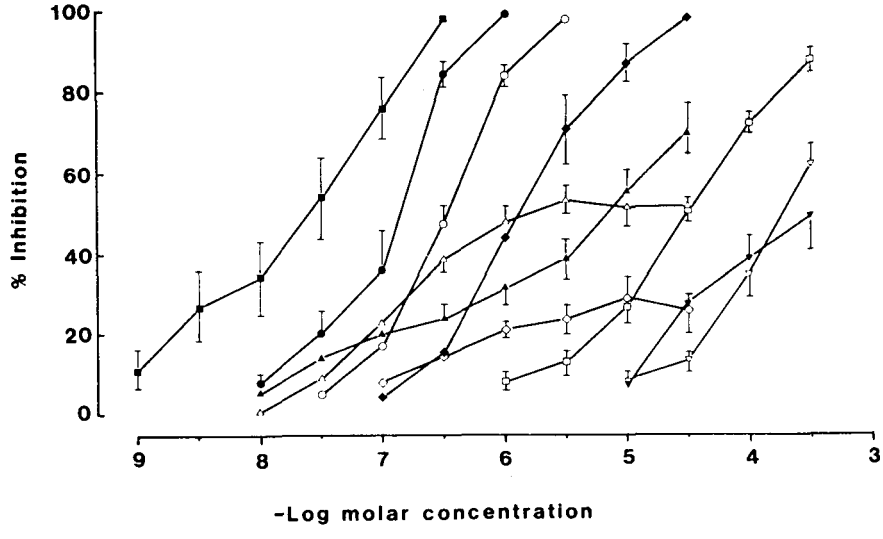

FIG. 1. Cumulative concentration-response curves for the inhibitory actions of sympathomimetic drugs on electrically-induced twitch contractions of guinea-pig isolated tracheal muscle: isoprenaline (■), adrenaline (●), noradrenaline (○), dopamine (□), terbutaline (◆), clonidine (△), oxymetazoline (▲), ephedrine (◇), lidamidine (▽) and WHR1370 (▼). Each point represents the mean ± s.e.m. Numbers of observations are shown in Table 1.

Table 1. Inhibitory potencies of sympathomimetic drugs on electrically induced contractions of guinea-pig isolated tracheal muscle with or without the propranolol (2 μM) treatment.

	Control			Propranolol (2 μM) treatment		
Drug	n	IC50 (−log M)[a]	% Maximum[b]	n	IC50 (−log M)[a]	% Maximum[b]
Noradrenaline	10	6·46 ± 0·06	98·2 ± 1·3	8	5·89 ± 0·12***	98·6 ± 1·1
Adrenaline	9	6·89 ± 0·12	99·2 ± 0·5	8	6·48 ± 0·06**	96·0 ± 1·4
Isoprenaline	10	7·66 ± 0·22	98·3 ± 1·1	6	5·14 ± 0·02***	95·4 ± 0·9
Dopamine	8	4·51 ± 0·07	88·0 ± 3·0	6	3·76 ± 0·16***	56·7 ± 4·7
Ephedrine	9	<3·52	28·8 ± 5·5	8	<3·52	35·9 ± 2·5
Terbutaline	9	5·82 ± 0·13	98·2 ± 1·0	8	3·56 ± 0·06***	55·4 ± 4·2
Clonidine	9	5·98 ± 0·17	53·5 ± 3·5	8	5·85 ± 0·15N.S.	53·4 ± 3·4
Oxymetazoline	9	5·29 ± 0·18	71·1 ± 6·3	8	5·53 ± 0·19N.S.	46·7 ± 4·2
Lidamidine	9	3·85 ± 0·19	62·5 ± 5·2	5	3·85 ± 0·15N.S.	62·6 ± 8·5
WHR1370	8	<3·52	49·4 ± 8·8	5	4·14 ± 0·14	73·6 ± 6·8

All values represent the mean ± s.e.m.
[a] −Log molar concentrations causing 50% reduction of the contraction height.
[b] % Maximum reduction of the contraction height obtained at the concentration ranges examined.
** $P < 0.01$; *** $P < 0.001$; N.S., not significant; these were compared with the control values using the unpaired Student's t-test.

was much less effective in inhibiting the electrically induced twitch contractions (Fig. 1 and Table 1). Among the sympathomimetic drugs tested, higher concentrations of oxymetazoline (10–30 μM), lidamidine (100–300 μM) and WHR1370 (100–300 μM) increased the basal tracheal tension by about 0·12–0·38 g.

When the twitch contractions were maximally inhibited by these drugs, responsiveness to exogenously supplied ACh (0·1–3 μM) were examined. The ACh-induced contractions, which were equipotent in amplitude with those evoked by electrical stimulation, were also inhibited largely ($P < 0.001$) by isoprenaline 0·3 μM, adrenaline 1 μM and dopamine 300 μM, and moderately ($P < 0.05$) by noradrenaline 3 μM and terbutaline 30 μM (Fig. 2). Clonidine 30 μM, lidamidine 300 μM and WHR1370 300 μM did not affect the ACh-induced contractions, while oxymetazoline 30 μM rather augmented the responses ($P < 0.05$) (Fig. 2).

The isoprenaline (0·3 μM)-induced twitch inhibition was mostly reversed by propranolol (2 μM) alone (Fig. 3a). Similar results were obtained against terbutaline (30 μM). However, the adrenaline (1 μM)-induced twitch inhibition was only partially reversed by propranolol (2 μM) alone, but was completely reversed by the addition of yohimbine (2 μM) (Fig. 3b). Similar antagonisms were also observed on the inhibitory responses to noradrenaline (3 μM) and dopamine (300 μM). On the other hand, twitch inhibitions induced by clonidine (2 μM), oxymetazoline (10 μM), lidamidine (300 μM) and WHR1370 (300 μM) were completely reversed by yohimbine (2 μM) alone.

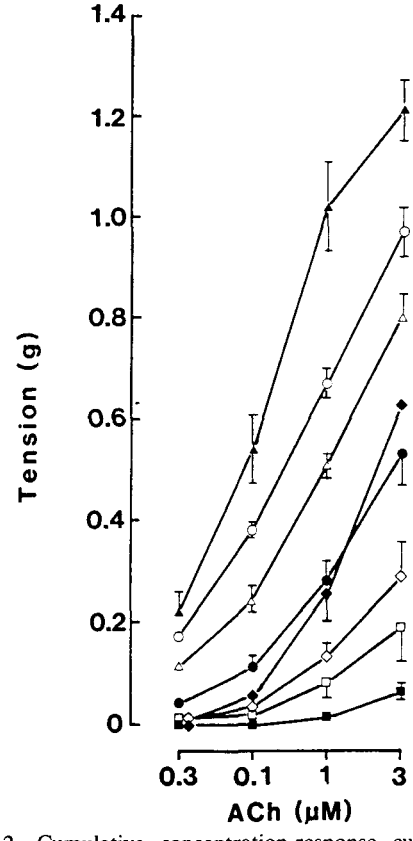

FIG. 2. Cumulative concentration-response curves for contractions to exogenous acetylcholine (ACh) of guinea-pig isolated tracheal muscle in the absence (control, ○, n = 17) and presence of isoprenaline 0·3 μM (■, n = 10), adrenaline 1 μM (□, n = 9), noradrenaline 3 μM (●, n = 10), dopamine 300 μM (◇, n = 9), terbutaline 30 μM (◆, n = 6), clonidine 30 μM (△, n = 9) or oxymetazoline 30 μM (▲, n = 9). The ordinate shows the developed tension (g) by ACh. Each point represents the mean ± s.e.m.

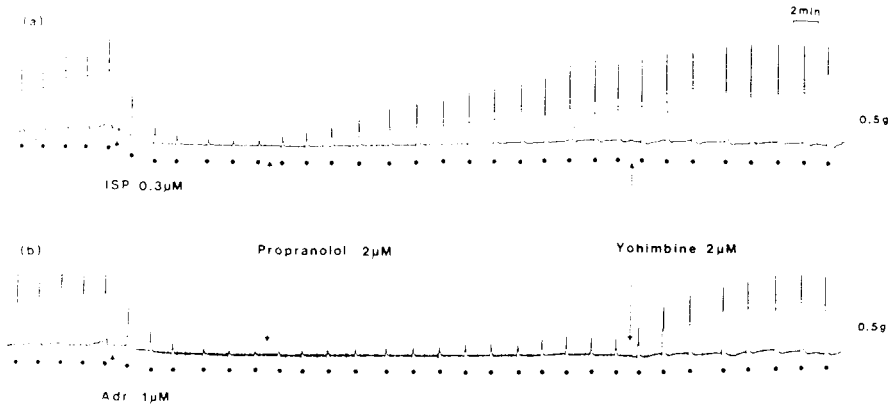

FIG. 3. The inhibitions by isoprenaline (ISP, 0·3 μM, a) and adrenaline (Adr, 1 μM, b) of the twitch contractions of guinea-pig isolated tracheal muscle evoked by electrical field stimulation (8 Hz, 0·5 ms, supramaximal voltage, 40 pulses) and its antagonism by propranolol (2 μM) alone and in the presence of yohimbine (2 μM). Vertical calibrations show 0·5 g tension developments of the tracheal muscle.

Inhibitory effects of sympathomimetic drugs on cholinergically mediated contractions of propranolol (2 μM)-treated tracheal muscle

In the presence of propranolol (2 μM), the concentration-response curve of isoprenaline for inhibiting the twitch contractions was largely shifted to the right (Fig. 4) and its IC50 value (−log M) decreased from 7·66 to 5·14 (Table 1). A reduced inhibitory response was also observed against terbutaline and its IC50 value decreased from 5·82 to 3·56. The rightward shifts of concentration-response curves of adrenaline, noradrenaline and dopamine by propranolol were less pronounced (Fig. 4). The decreases of each IC50 value were within 0·41–0·75, but these were statistically significant (Table 1). On the other hand, twitch inhibitory actions of clonidine, oxymetazoline, lidamidine and WHR1370 were not significantly modified by the propranolol treatment, instead they were augmented (Fig. 4 and Table 1). Again, ephedrine was much less effective in inhibiting the twitch even in the presence of propranolol. Basal tension of the propranolol-treated tracheal muscle was increased about 0·12–0·38 g by higher concentrations of adrenaline (1–10 μM), noradrenaline (10–30 μM), dopamine (100–300 μM), clonidine (10–30 μM), oxymetazoline (10–30 μM), lidamidine (100–300 μM) and WHR1370 (100–300 μM). Contractions to exogenous ACh (0·1–3 μM) were largely ($P < 0·001$) inhibited by isoprenaline 30 μM, slightly ($P < 0·05$) augmented by oxymetazoline (30 μM), but not significantly modified by adrenaline (10 μM), noradrenaline (30 μM), dopamine (300 μM), clonidine (30 μM), terbutaline (300 μM), lidamidine (300 μM) and WHR1370 (300 μM) (Fig. 5).

Twitch inhibitory actions of adrenaline (10 μM), noradrenaline (30 μM), dopamine (300 μM), clonidine (30 μM), lidamidine (300 μM) and WHR1370 (300 μm) were mostly reversed by yohimbine (2 μM) alone, whereas those of isoprenaline (30 μM) and terbutaline (300 μM) were reversed by further addition of propranolol (20 μM).

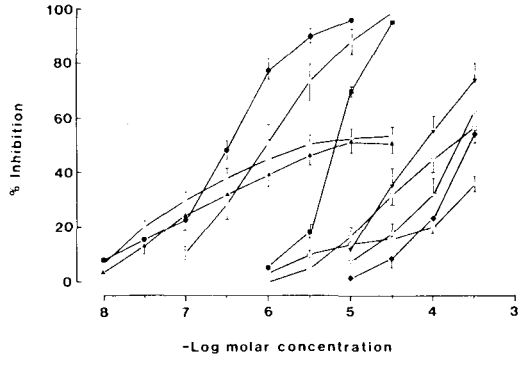

FIG. 4. Cumulative concentration-response curves for the inhibitory actions of sympathomimetic drugs on electrically-induced twitch contractions of guinea-pig isolated tracheal muscle in the presence of propranolol (2 μM): adrenaline (●), noradrenaline (○), isoprenaline (■), dopamine (□), clonidine (△), oxymetazoline (▲), terbutaline (◆), WHR1370 (▼),, lidamidine (▽) and ephedrine (◇). Each point represents the mean ± s.e.m. Numbers of observations are shown in Table 1.

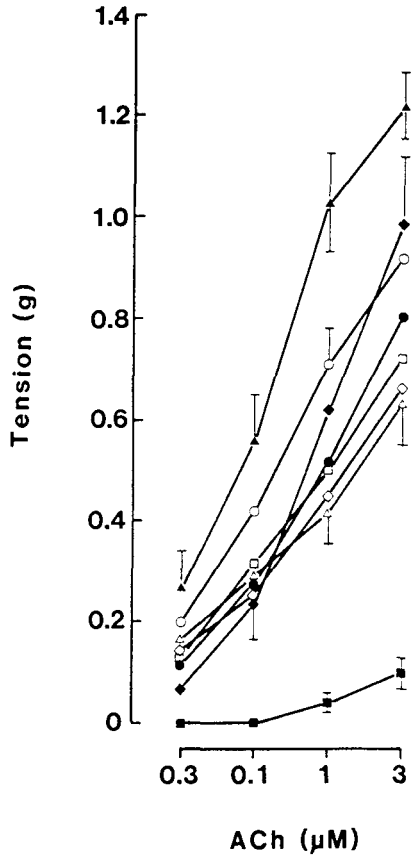

FIG. 5. Cumulative concentration-response curves for contractions to exogenous acetylcholine (ACh) of propranolol (2 μM)-treated guinea-pig tracheal muscle in the absence (control, ○, n = 14) and presence of isoprenaline 30 μM (■, n = 5), adrenaline 10 μM (□, n = 6), noradrenaline 30 μM (●, n = 10), dopamine 300 μM (◇, n = 5), clonidine 30 μM (△, n = 5), oxymetazoline 30 μM (▲, n = 5) or terbutaline 300 μM (◆, n = 5). The ordinate shows the developed tension (g) by ACh. Each point represents the mean ± s.e.m.

DISCUSSION

Previously, we have reported that exogenous noradrenaline can inhibit cholinergically mediated contractions of guinea-pig isolated tracheal muscle, preferentially via prejunctional α_2-adrenoceptors and partly via postjunctional β_2-adrenoceptors (Kamikawa 1982). Similar observations have also been made by other investigators (Grundström et al 1981; Visnovsky et al 1982). In the present experiments, all the sympathomimetic drugs tested also inhibited electrically induced, cholinergically mediated contractions of the normal guinea-pig tracheal muscle. The order of potency was isoprenaline > adrenaline > noradrenaline > clonidine = terbutaline > oxymetazoline > dopamine > lidamidine > WHR1370 and, much weaker, ephedrine, on the basis of IC50 values (Table 1). However, the order was changed to the following under β-adrenoceptor blockade by propranolol: adrenaline > noradrenaline > clonidine = oxymetazoline > isoprenaline > WHR1370 > lidamidine > dopamine > terbutaline and, much weaker, ephedrine. The different orders of potency can be explained by the different affinities of these drugs for both α_2- and β_2-adrenoceptors. Inhibitory actions of adrenaline, noradrenaline and dopamine seem to be preferentially mediated by prejunctional α_2-adrenoceptors and partly by postjunctional β_2-adrenoceptors, because these were mostly prevented by yohimbine but partly by propranolol, and in the presence of propranolol, these catecholamines can inhibit the electrically-induced contraction without significant modification on the ACh-induced one. On the other hand, inhibitory actions of isoprenaline and terbutaline were mostly mediated by postjunctional β_2-adrenoceptors, because these were antagonized by propranolol alone. All the selective α_2-stimulants, clonidine, oxymetazoline, lidamidine and WHR1370, inhibited the electrically induced contractions, but not the ACh-induced one, probably via prejunctional α_2-adrenoceptors. These results confirmed our previous hypothesis that cholinergic neurotransmission in airway smooth muscles can be inhibited not only by postjunctional β_2-adrenoceptors but also by prejunctional α_2-adrenoceptors (Kamikawa 1982). Prejunctional α_2-adrenoceptors are located on both sympathetic adrenergic and parasympathetic cholinergic nerve terminals (Starke 1981). Noradrenaline and adrenaline can stimulate both α_2-adrenoceptors as full agonists, while clonidine and oxymetazoline act as partial agonists on the receptors (Ruffolo 1984). Newly developed α_2-agonists, lidamidine and WHR1370, can selectively stimulate α_2-adrenoceptors located on cholinergic nerves in the gastrointestinal tract, and therefore are useful for treating ulcers and diarrhoea (DiJoseph et al 1984). However, these drugs had less potency and efficacy than catecholamines in inhibiting cholinergic neurotransmission of the trachea preparation. This suggests that α_2-adrenoceptors located on airway cholinergic nerves may be different in nature from those in the gut.

Selective β_2-stimulants are widely used in bronchial asthma, but refractoriness or desensitization by prolonged use limits their clinical usefulness.

Recently, Olsson & Ekdahl (1985) demonstrated that inhaled clonidine more effectively inhibited a vagally mediated bronchospasm in anaesthetized guinea-pigs than atropine. In addition, Davis & Lieberman (1982) have reported that platelet α_2-adrenergic responses were normal in asthmatic subjects. Since it is well known that airway cholinergic nerves play an important role in the bronchial hyperreactivity of asthma (Boushey 1985), these findings indicate that selective α_2-stimulants acting on airway cholinergic nerves might be useful for preventing bronchospasms of asthmatic patients with β-adrenoceptor blockade. Although none of the selective α_2-stimulants had sufficient potency and efficacy to inhibit cholinergic neurotransmission in the present experiments, it is likely that potent α_2-stimulants will become available as a new class of antiasthmatic drugs.

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