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Inhibitory effects of catecholamines on cholinergically and non-cholinergically mediated contractions of guinea-pig isolated bronchial muscle

YUICHIRO KAMIKAWA, YASUO SHIMO, *Department of Pharmacology, Dokkyo University School of Medicine, Mibu, Tochigi 321-02, Japan*

Abstract—The actions of catecholamines on the responses evoked by electrical field stimulation or by acetylcholine and substance P in guinea-pig bronchial strip chain have been examined. Electrical field stimulation evoked a biphasic contraction, consisting of a cholinergically-mediated fast contraction followed by a non-cholinergically-mediated slow contraction. All catecholamines tested caused a concentration-dependent reduction in the height of the biphasic contraction, where non-cholinergic contractions were more potently inhibited. The inhibitory effect of isoprenaline was largely prevented by propranolol (2 μM) alone, whereas those of noradrenaline and adrenaline were prevented by treatment with both propranolol (2 μM) and yohimbine (2 μM). The inhibitory effect of dopamine was unaffected either by propranolol (2 μM), yohimbine (2 μM) or haloperidol (10 μM). Submaximal contractions of bronchial muscle evoked by exogenous acetylcholine (2 μM) or substance P (0·2 μM) were also inhibited by catecholamines, except dopamine, but the effects were antagonized by propranolol (2 μM) alone. The results suggest that in guinea-pig isolated bronchial muscle, catecholamines can inhibit both cholinergic and non-cholinergic excitatory neurotransmissions not only by postjunctional β-adrenoceptors but also by prejunctional α_2-adrenoceptors.

Previously, we have reported that catecholamines and related symphatomimetic amines can inhibit cholinergic neurotransmission of the guinea-pig trachea not only via postjunctional β_2-adrenoceptors but also by prejunctional α_2-adrenoceptors (Kamikawa & Shimo 1986). Similar observations were made by Vermeire & Vanhoutte (1979) and Grundström et al (1981). Recent evidence indicates that guinea-pig peripheral airways are predominantly innervated by excitatory cholinergic and non-cholinergic nerves and that substance P (SP) or related tachykinins might function as the transmitter substance of the non-cholinergic nerves (Håkanson et al 1983; Lundberg et al 1983; Leander et al 1984; Andersson & Grundström 1987; Kamikawa & Shimo 1989). Hence, in the present study we have investigated the modulating effects of catecholamines on neurogenic contractions of the guinea-pig bronchi. A preliminary report of some of these results has been made (Kamikawa & Shimo 1987).

Correspondence to: Y. Kamikawa, Department of Pharmacology, Dokkyo University School of Medicine, Mibu, Tochigi 321-02, Japan.

Materials and methods

Male guinea-pigs (300 to 700 g) were stunned, the tracheobronchial tree excised and the bronchial strip chain prepared (Kamikawa & Shimo 1989). Briefly, two pieces of right and left bronchial 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 11 (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 bronchial response was isometrically recorded by means of a force-displacement transducer (Nihon Kohden SB-IT-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-1011). The total number of stimulating pulses was kept constant at 40. For the elimination of endogenous prostaglandin biosynthesis in response to field stimulation, the Krebs solution contained 2 μM indomethacin. When the strip was electrically stimulated, a biphasic contraction was obtained composed of an initial fast contraction mediated by cholinergic- followed by a sustained contraction mediated by non-cholinergic-nerve stimulation (Kamikawa & Shimo 1989). The heights of these contractions were comparable to those of submaximal contractions induced by exogenous acetylcholine (ACh, 2 μM) and SP (0·2 μM), respectively. The effects of catecholamines on the electrically induced contractions were measured as the percentage changes of the original contraction height obtained just before the drug was applied to the bath.

Drugs used were noradrenaline bitartrate, adrenaline bitartrate, isoprenaline bitartrate, yohimbine hydrochloride, propranolol hydrochloride, carbachol chloride (Sigma), indomethacin (Sankyo), dopamine hydrochloride (Wako), acetylcholine chloride (Dai-ichi), substance P (Peptide Institute) and haloperidol (Searle). To prepare the drug solutions, catecholamines were

dissolved in and diluted with 0·9% w/v NaCl (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 in this paper refer to the final bath concentrations.

Results

All catecholamines tested inhibited the electrically induced biphasic contraction of guinea-pig bronchial muscle. The responses were concentration-dependent and reversible by washing. However, their inhibitory potencies were variable not only among catecholamines but also between cholinergic and non-cholinergic components of the contraction, and isoprenaline was the most potent (Fig. 1). The inhibitory actions of isoprenaline, adrenaline and noradrenaline on non-cholinergically mediated contractions were about five times more potent than those on cholinergically mediated contractions on the basis of each concentration required to inhibit the contraction height by 50% (IC50 in Table 1). Dopamine showed a selective inhibition on the non-cholinergically mediated contractions (Fig. 1, Table 1).

Table 1. Inhibitory potencies of catecholamines on electrically induced biphasic contractions of guinea-pig isolated bronchial muscle with or without propranolol (2 μM) treatment.

	Control		Propranolol (2 μM) treatment	
Catecholamines	n	IC50 (-log M)[a]	n	IC50 (-log M)[a]
A. Cholinergically mediated contraction				
Noradrenaline	9	5·34 ± 0·07	7	4·93 ± 0·08**
Adrenaline	7	6·37 ± 0·21	6	5·63 ± 0·07**
Isoprenaline	7	6·47 ± 0·15	6	4·92 ± 0·08***
Dopamine	6	< 3·5	6	4·34 ± 0·23
B. Non-cholinergically mediated contraction				
Noradrenaline	7	6·02 ± 0·13	7	5·84 ± 0·12 NS
Adrenaline	6	6·97 ± 0·20	6	6·30 ± 0·10*
Isoprenaline	6	7·21 ± 0·15	6	5·30 ± 0·08***
Dopamine	6	4·96 ± 0·12	6	5·02 ± 0·15 NS

All values represent the mean ± s.e.m. a, -log concentrations causing 50% reduction of the contraction height. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; N.S., not significant.

These were compared with control values using the unpaired t-test.

The isoprenaline (1 μM)-induced inhibition of the electrically induced biphasic contraction was mostly reversed by propranolol (2 μM) alone (Fig. 2C). However, the inhibitory actions of noradrenaline (10 μM) and adrenaline (1 μM) were only partially reversed by propranolol (2 μM) alone, but were mostly reversed by the further addition of yohimbine (2μM) (Fig. 2A, B). The inhibitory action of dopamine was unaffected by these antagonists (Fig. 2D) and by haloperidol (10 μM, n = 3). In the presence of propranolol (2 μM), the concentration-response curve of isoprenaline for inhibiting the electrically induced biphasic contraction was extensively shifted to the right and its IC50 value (-log M) decreased from 6·47 to 4·92 for the cholinergic component and from 7·21 to 5·30 for the non-cholinergic component (Table 1). The rightward shift of concentration-response curves of noradrenaline and adrenaline by propranolol were less pronounced and the decreases of each IC50 value were within 0·2–0·7 (Table 1). In contrast, the inhibitory action of dopamine was augmented by propranolol (Table 1).

When the electrically induced contractions were maximally inhibited by catecholamines, responsiveness to exogenously supplied ACh (2 μM) and SP (0·2 μM) were examined. Each concentration of ACh and SP produced a submaximal contraction of the bronchial muscle, which was equal in amplitude with the electrically induced cholinergic and non-cholinergic contractions, respectively (Kamikawa & Shimo 1989). In contrast to the actions on the electrically induced response, noradrenaline (10 μM), adrenaline (1 μM) and isoprenaline (1 μM) inhibited the ACh-included contraction more than the SP-induced contraction, but dopamine (300 μM) did not significantly inhibit either contraction (Fig. 3). These inhibitory actions were fully reversed by propranolol (2 μM) alone (Fig. 3B).

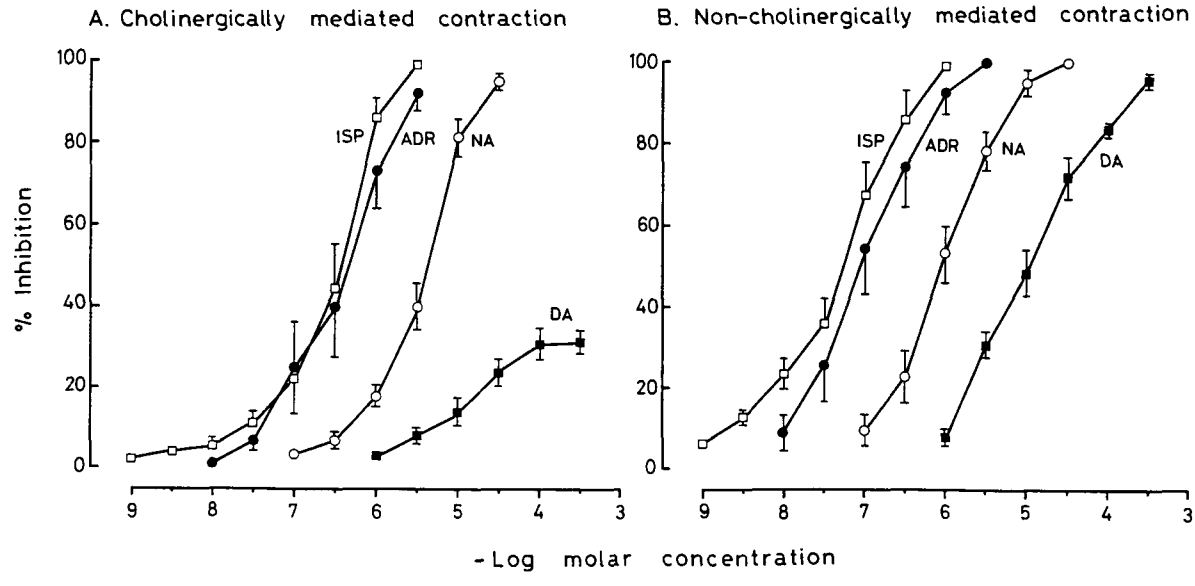

FIG. 1. Cumulative concentration-response curves for the inhibitory actions of catecholamines on electrically induced biphasic contractions of guinea-pig isolated bronchial muscle: isoprenaline (ISP, □), adrenaline (ADR, ●), noradrenaline (NA, ○) and dopamine (DA, ■). A, effects on cholinergically mediated contraction; B, effects on non-cholinergically mediated contraction. Each point represents the mean ± s.e.m. Numbers of observations are shown in Table 1.

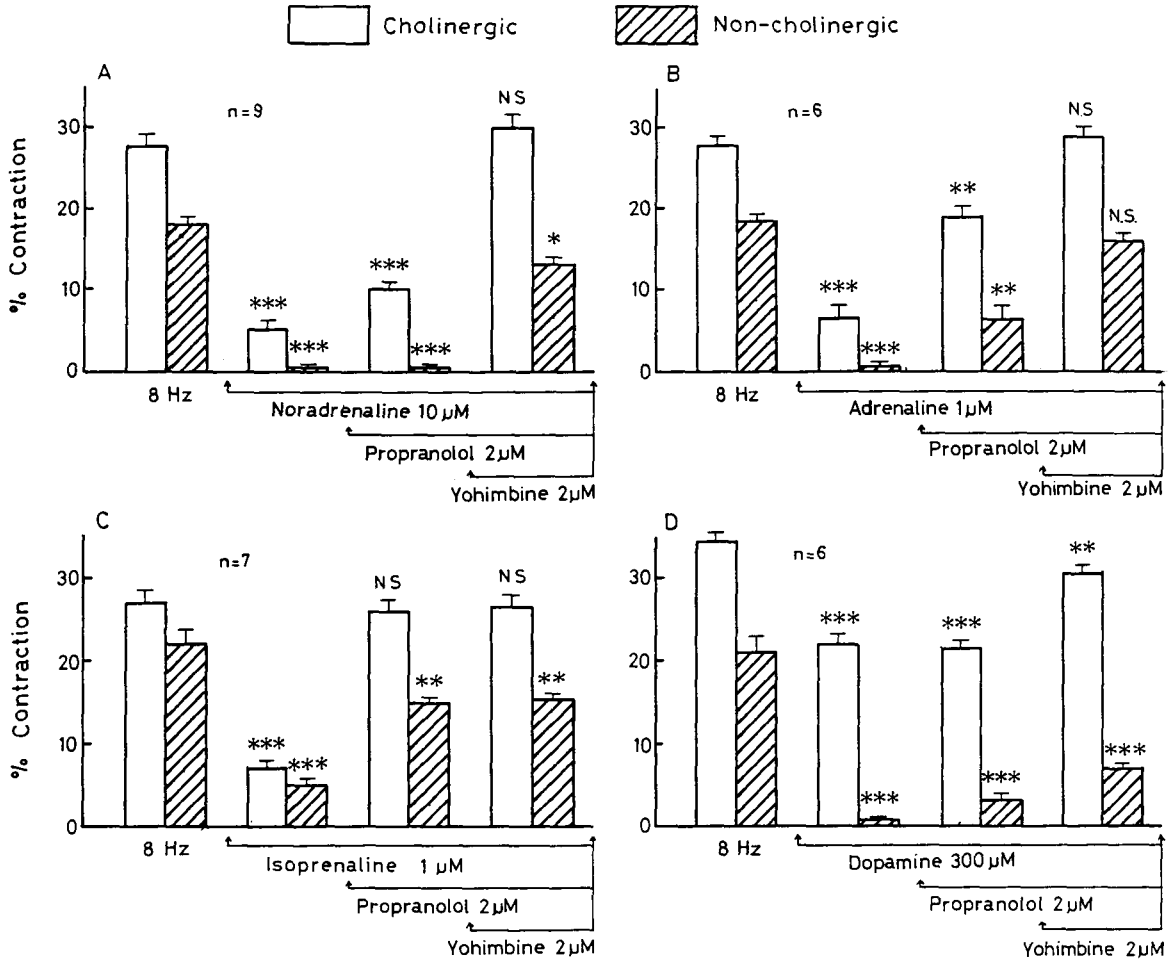

FIG. 2. Effect of propranolol (2 μM) and yohimbine (2 μM) on the inhibitory actions of catecholamines on electrically induced cholinergic (open column) and non-cholinergic (shaded column) contractions of guinea-pig isolated bronchial muscle. These antagonists were applied to the tissue after maximal inhibition by each concentration of catecholamines: A, noradrenaline 10 μM; B, adrenaline 1 μM; C, isoprenaline 1 μM; D, dopamine 300 μM. Vertical bars in each column show the standard error of mean value. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; N.S., not significant. These were compared with the original contraction height evoked by 8 Hz electrical stimulation using the paired t-test. Ordinate scales show the percentage response of the maximum contraction induced by carbachol 10 μM.

Discussion

In this study we have demonstrated that catecholamines inhibit the electrically induced neurogenic contractions of guinea-pig bronchial muscle. The order of potency was isoprenaline > adrenaline > noradrenaline > dopamine. Catecholamines more strongly inhibited the non-cholinergic component than the cholinergic component of the electrically induced response. However, these catecholamines inhibited submaximal contractions to exogenous ACh or SP less than those to electrical stimulation. This suggests that their inhibitory effects on the electrically induced response are mediated not only by postjunctional effects on bronchial muscle but also by prejunctional reduction of the transmitter release from autonomic nerves. The inhibitory action of isoprenaline was mostly mediated by postjunctional β-adrenoceptors, because isoprenaline simultaneously inhibited both electrically- and exogenous ACh- or SP-induced contractions and the actions were fully antagonized by propranolol alone. The inhibitory actions of adrenaline and noradrenaline seem to be preferentially mediated by prejunctional α_2-adrenoceptors and partly by postjunctional β-adrenoceptors, because these more effectively inhibited the electrically induced response than the exogenous ACh- or SP-induced response and these actions were mostly prevented by yohimbine in the presence of propranolol as well as partly by propranolol alone. Dopamine showed the least potency among catecholamines in inhibiting the neurogenic contractions, but produced a selective inhibition on the non-cholinergic component. The inhibitory action seems to be preferentially mediated by a prejunctional mechanism, because dopamine did not inhibit the exogenous SP-induced contraction. However, the type of dopamine receptor remains to be elucidated, because the action was not significantly modified by yohimbine, propranolol or haloperidol. These results indicate that catecholamines can inhibit excitatory cholinergic and non-cholinergic neurotransmissions in guinea-pig bronchial muscle not only via prejunctional mechanisms but also via postjunctional mechanisms. Inhibitory effects of catecholamines on cholinergic neuro-transmission have already been reported on guinea-pig tracheal muscle (Grundström et al 1981; Kamikawa & Shimo 1986), but their potencies were greater than those observed by us on guinea-pig bronchial muscle. In contrast, catecholamines did not modify the non-adrenergically-mediated relaxation of guinea-pig tracheal muscle (Kamikawa 1987), but potently inhibited the non-

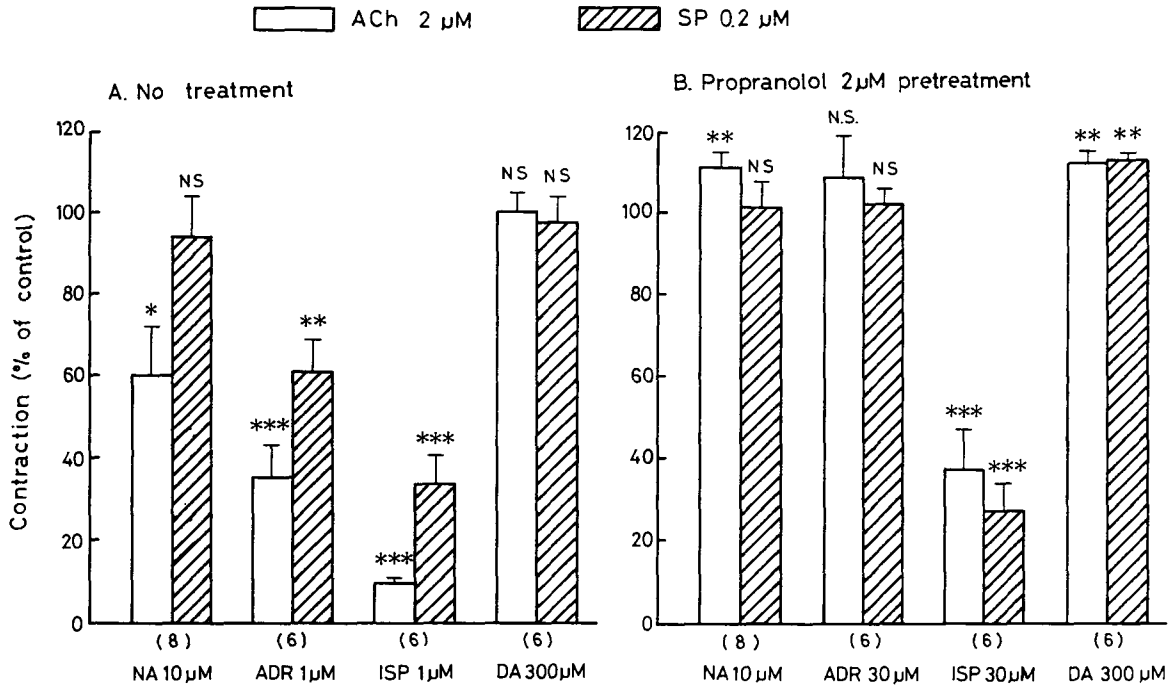

FIG. 3. Effects of catecholamines on submaximal contractions to exogenously supplied acetylcholine (ACh 2 μM, open column) and substance P (SP 0·2 μM, shaded column) of guinea-pig isolated bronchial muscle in the absence (A) or presence (B) of pretreatment with propranolol (2 μM). NA, noradrenaline 10 μM; ADR, adrenaline 1 μM; ISP, isoprenaline 1 μM; DA, dopamine 300 μM. Ordinate scales show the percentage changes of the ACh- or SP-induced contraction by catecholamines. Vertical lines in each column show the s.e.m. Numbers in parenthesis under each column show the numbers of observations. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; NS, not significant. These were compared with control response using the paired t-test.

cholinergically-mediated contraction of guinea-pig bronchial muscle. These findings indicate that neuromodulating effects of catecholamines are different between proximal and peripheral airways. Although the transmitter substance of non-cholinergic nerves is not yet determined, much evidence suggests that SP or a related tachykinin might function as the transmitter (Håkanson et al 1983; Lundberg et al 1983; Leander et al 1984; Kamikawa & Shimo 1989). The tachykinin-containing nerves in peripheral airways are thought to be sensory nerves in origin (Andersson & Grundström 1987; Lundberg & Saira 1987). These sensory nerves function not only as a primary sensory pathway in the central nervous system but also as mediators of the antidromic axon reflex. Since the pulmonary axon reflex is considered to contribute to the pathophysiology of bronchial asthma (Barnes 1987), the present findings indicate that catecholamines may exhibit their anti-asthmatic effects via the interference with the axon reflex mechanism in peripheral airways.

In conclusion, catecholamines can inhibit both cholinergically and non-cholinergically mediated contractions of guinea-pig bronchial muscle via prejunctional and/or postjunctional mechanisms.

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