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INHIBITORY ACTIONS OF CATECHOLAMINES ON ELECTRICALLY INDUCED CONTRACTIONS OF THE SUBMUCOUS PLEXUS-LONGITUDINAL MUSCULARIS MUCOSAE PREPARATION OF THE GUINEA-PIG OESOPHAGUS

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1 The submucous plexus-longitudinal muscularis mucosae preparation of the guinea-pig oesophagus was used to study the actions of catecholamines on the twitch responses to electrical stimulation.

2 When the preparation was stimulated coaxially (0.1 Hz, 0.5 ms, supramaximal voltage), stable twitch-like contractions were obtained. These were abolished by tetrodotoxin (0.1 μM) and atropine (0.1 μM), potentiated by physostigmine (0.1 μM), and were mediated presumably by stimulation of intramural cholinergic nerves.

3 The twitch contractions of the muscularis mucosae were inhibited by catecholamines, in a concentration-dependent manner. The order of potency was isoprenaline > adrenaline > noradrenaline > dopamine.

4 The inhibitory actions of noradrenaline (1 μM) and adrenaline (1 μM) were partly reversed by phentolamine (1 μM) or by propranolol (1 μM), and completely abolished by both antagonists together. The inhibitory effect of dopamine (300 μM) was largely reversed by phentolamine (1 μM), but not by propranolol (1 μM), while the inhibitory action of isoprenaline was competitively antagonized only by propranolol (pA_2 of 7.6).

5 The contraction of the muscularis mucosae to exogenously applied acetylcholine (ACh, 20 nM) which was comparable in magnitude with that to electrical stimulation was also inhibited by isoprenaline (0.1 μM), adrenaline (1 μM) and noradrenaline (1 μM), but not by dopamine (300 μM). In the presence of propranolol (1 μM), noradrenaline, adrenaline and dopamine potentiated the ACh-induced contraction, while the effect of isoprenaline was mainly antagonized. The potentiating effects were antagonized by further treatment with phentolamine (1 μM).

6 Adrenaline, noradrenaline and dopamine but not isoprenaline, produced a weak contraction of the longitudinal muscularis mucosae in the presence of propranolol (3 μM). The contractile responses were completely inhibited by phentolamine (3 μM). Tone in the muscularis mucosae induced by carbachol (3 μM) in the presence of phentolamine (10 μM) was inhibited by catecholamines, in a concentration-dependent manner, an effect that was competitively antagonized by propranolol.

7 In the submucous plexus-longitudinal muscularis mucosae preparation of the guinea-pig oesophagus there are three types of adrenoceptor, inhibitory prejunctional α-adrenoceptors, excitatory postjunctional α-adrenoceptors and inhibitory postjunctional β-adrenoceptors, and cholinergic neurotransmission is inhibited by catecholamines acting at both prejunctional α- and postjunctional β-adrenoceptors.

Introduction

The motility of the mammalian gastrointestinal tract is regulated not only by extrinsic sympathetic and parasympathetic nerves, but also by the intrinsic nervous system. The intrinsic nervous system contains sensory and motor (excitatory and inhibitory) neurones and interneurones. The motor neurones consist of cholinergic, adrenergic, and non-adrenergic non-cholinergic (NANC) neurones. The cell bodies of these neurones are arranged in two intramural plexuses, the myenteric plexus which is located between the longitudinal and circular smooth muscle layers, and the submucous plexus which is located between the circular muscle and the muscularis mucosae. The cholinergic neurones act directly on the intestinal smooth muscle and their stimulation produces a contraction via muscarinic receptors.

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They are also involved in the excitatory component of the peristaltic reflex. On the other hand, postganglionic sympathetic nerves running to the intestine through the mesentery inhibit peristalsis by releasing noradrenaline (NA) which acts on cholinergic neurones to reduce the output of acetylcholine (ACh) (Furness & Costa, 1980). By use of fluorescence histochemistry, it has been demonstrated that the majority of adrenergic nerve terminals end on the ganglion cells of enteric plexuses, and that the intestinal smooth muscles are innervated only sparsely by adrenergic nerves (Norberg, 1964; Hollands & Vanov, 1965; Jacobowitz, 1965; Costa & Furness, 1973). Exogenously applied catecholamines or sympathetic nerve stimulation inhibit cholinergic neurotransmission in the intestine via prejunctional α-adrenoceptors (Paton & Vizi, 1969; Kosterlitz, Lydon & Watt, 1970; Gillespie & Khoyi, 1977; Wikberg, 1977; 1978; Drew, 1978; Kamikawa & Shimo, 1978; Bauer, 1981). Most of these studies have been performed on the myenteric plexus rather than on the submucous plexus. The muscularis mucosae of the guinea-pig oesophagus is innervated chiefly by excitatory cholinergic nerves and very sparsely with inhibitory adrenergic nerves; there are no NANC nerves (Kamikawa & Shimo, 1979). The isolated muscularis mucosae of the guinea-pig oesophagus contains a submucous plexus including cholinergic nerve cell bodies, independent of the myenteric plexus. This led us to examine the effects of catecholamines on cholinergic neurotransmission in the submucous plexus of the guinea-pig oesophagus measured as their effects on the twitch-like contractions of the longitudinal muscularis mucosae induced by electrical stimulation of intramural cholinergic nerves. The results indicate that catecholamines inhibit the twitch via both prejunctional α- and postjunctional β-adrenoceptors. Preliminary reports of some of these results have been made (Kamikawa, Uchida & Shimo, 1981 a, b).

Methods

Male guinea-pigs (300 to 500 g) were stunned, the oesophagus excised and the isolated muscularis mucosae attached to the submucous plexus was prepared (Kamikawa & Shimo, 1979). Briefly, the excised oesophagus was pinned on a cork mat immersed in Tyrode solution. The outer striated muscle coat was cut longitudinally, and gently peeled away leaving an inner tube. The tube including longitudinal muscularis mucosae, about 15 mm long without a load, was immersed in a 15 ml organ bath filled with Tyrode solution of the following composition (mM); NaCl 136.8, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.05, $NaHCO_3$ 11.9, NaH_2PO_4 0.42, disodium edetate (EDTA) 0.03, ascorbic acid 0.12, and glucose 5.56 (pH 7.4). The Tyrode solution always contained 20 μM choline chloride and was bubbled with 5% CO_2 and 95% O_2, and maintained at 37°C. The preparation was suspended under a 0.3 g load and 60 min was allowed to elapse before experiments were started. Responses of the longitudinal muscularis mucosae were recorded isotonically by means of an isotonic transducer (MEC-1411) and a Nihon Kohden Polygraph recorder (RJG-4004). Isotonic responses of this tissue were amplified about ten times on a recording chart using a polygraph and hence very small contractures could be recorded.

Electrical stimulation of intramural nerves in the muscularis mucosae was carried out transmurally by means of two coaxial platinum electrodes, the anode in the lumen and the cathode in the organ bath. The stimulation parameters were always 0.1 Hz, 0.5 ms and supramaximal voltage (approx. 40V). When the strip was electrically stimulated, stable twitch-like contractions were obtained, the height of which was nearly equivalent to that of the contraction caused by exogenously applied ACh (20 nM). The inhibition of the twitch contractions by catecholamines was measured as the percentage inhibition of the original twitch height obtained just before the drug was applied to the bath. In experiments to obtain cumulative concentration-response curves, the intervals between doses were approx. 60 min. The direct action of catecholamines on the longitudinal muscularis mucosae was also examined in the absence of electrical stimulation. Since there are both α-excitatory and β-inhibitory adrenoceptors in this tissue (Bailey, 1965), the α-action was examined in the presence of propranolol (3 μM), and the β-action on the carbachol (3 μM)-contracted preparation in the presence of phentolamine (10 μM). The concentrations of catecholamines required to inhibit the twitch response by 50% (IC_{50}) or to relax the maximum contraction produced by carbachol by 50% (EC_{50}) were calculated from individual concentration-response curves and analysed statistically. The pA_2 values of the antagonists were calculated by the method of Arunlakshana & Schild (1959). Statistical significances were determined by Student's t test.

Drugs used were (−)-noradrenaline bitartrate, (−)-adrenaline bitartrate, (−)-isoprenaline hydrochloride, carbachol chloride (Sigma), dopamine hydrochloride, physostigmine sulphate, atropine sulphate (Wako), (±)-propranolol hydrochloride, tetrodotoxin (Sankyo), phentolamine mesylate (Ciba-Geigy), acetylcholine chloride (Daiichi), papaverine hydrochloride and haloperidol (Dainippon). To prepare stock solutions, catecholamines were dissolved in 0.9% w/v NaCl solution (saline) containing 20 μM ascorbic acid; all other drugs were dissolved in normal saline. Further dilutions were made with Tyrode

solution each day. The molar concentrations of drugs described in this paper refer to the final bath concentrations.

Results

Response of the oesophageal muscularis mucosae to electrical stimulation

The isolated muscularis mucosae of the guinea-pig oesophagus usually showed neither tone nor spontaneous activity. When electrical stimulation (0.1 Hz, 0.5 ms, supramaximal voltage) was applied to the muscularis mucosae, very stable twitch-like contractions were obtained, the height of which was about 50% of the maximum contraction caused by high frequency electrical stimulation (20 Hz, 60 pulses; Kamikawa & Shimo, 1979). The twitch contractions were mediated by stimulation of intramural cholinergic nerves in the muscularis mucosae, since they were completely inhibited by tetrodotoxin (0.1 μM, $n = 20$) or atropine (0.1 μM, $n = 12$) and were potentiated by about 20% by physostigmine (0.1 μM, $n = 5$).

Effects of catecholamines on the electrically-induced twitch contractions

All the catecholamines tested inhibited the contractions of the isolated muscularis mucosae induced by transmural electrical stimulation (Figure 1). Above 0.01 μM, NA inhibited and at 1 μM abolished the contractions. The twitch remained abolished in the presence of NA, but was immediately restored after washout. Figure 2 shows cumulative concentration-response curves for the inhibitory actions of catecholamines. Isoprenaline (Isop) and adrenaline (Adr) produced similar inhibitions to those produced by NA but were more potent, while dopamine was much less effective. The relative potency of Isop, Adr and dopamine compared with that of NA (= 1) were 7.3, 2.7 and 0.001, respectively, on the basis of each IC_{50} value in Table 1. The NA and Adr (1 μM)-induced inhibitions were only partially reversed by phentolamine (1 μM), but were completely reversed by the additional application of propranolol (1 μM) (Figure 1). Similar results were obtained when the

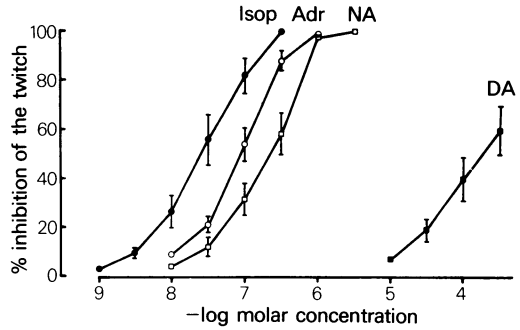

Figure 2 Cumulative concentration-response curves for the inhibitory actions of catecholamines on the electrically induced twitch contractions of the isolated muscularis mucosae of the guinea-pig oesophagus: isoprenaline (Isop, ●, $n = 14$); adrenaline (Adr, ○, $n = 13$); noradrenaline (NA, □, $n = 15$); dopamine (DA, ■, $n = 8$). All catecholamines inhibited the twitch contractions, in a concentration-dependent manner; the order of potency was isoprenaline > adrenaline > noradrenaline > dopamine. Each point represents the mean response; vertical lines show s.e.mean.

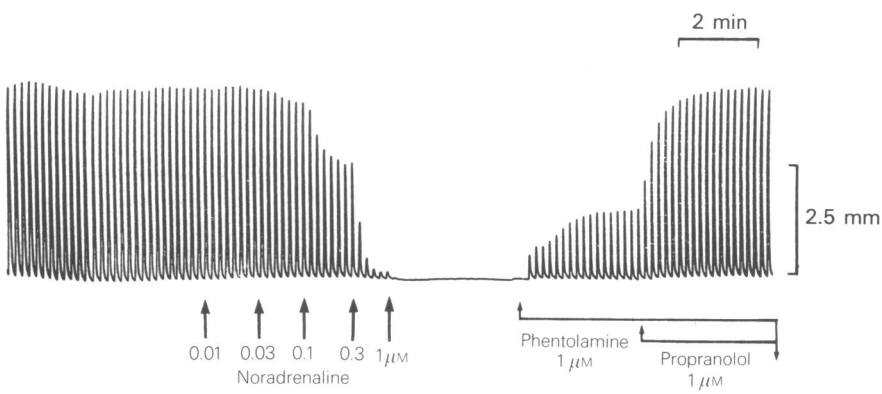

Figure 1 The inhibition, by cumulatively applied noradrenaline, of the twitch contractions of the isolated muscularis mucosae of the guinea-pig oesophagus induced by electrical stimulation (0.1 Hz, 0.5 ms, supramaximal voltage) and its antagonism by phentolamine (1 μM) alone and in the presence of propranolol (1 μM). The reversal, by phentolamine alone, of the twitch inhibition was incomplete; reversal was completed by further addition of propranolol. Vertical calibration shows 2.5 mm shortening of the tissue.

Table 1 50% inhibition (IC_{50}) of electrically-induced twitch contractions of the isolated muscularis mucosae of the guinea-pig oesophagus

Treatment	*IC_{50} (μM, mean ± s.e. mean)*			
	Noradrenaline	*Adrenaline*	*Isoprenaline*	*Dopamine*
Control	0.26 ± 0.05 (15)	0.094 ± 0.013 (13)	0.035 ± 0.010 (14)	194 ± 56.3 (8)
Propranolol (3 μM)	0.99 ± 0.28 (14)*	0.21 ± 0.06 (15)^{NS}	2.33 ± 0.35 (15)***	59.8 ± 23.5 (12)*
Phentolamine (1 μM)	0.37 ± 0.13 (16)^{NS}	1.34 ± 0.49 (16)**	0.022 ± 0.011 (12)^{NS}	1315 ± 190 (12)**

Numbers in parentheses show numbers of observations. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ^{NS}, not significant.

order of application of these antagonists was reversed. However, the Isop (0.1 μM)-induced twitch inhibition was reversed completely by propranolol (1 μM) alone, while that induced by dopamine (300 μM) was almost completely reversed by phentolamine (1 μM) alone. A similar pattern of antagonism of the inhibitory actions of catecholamines on electrically-induced contractions was also observed following pretreatment of the tissue with propranolol or phentolamine (Table 1). The IC_{50} value for NA was not significantly affected by pretreatment with phentolamine (1 μM), but was increased about 4 times by propranolol (3 μM) treatment. The value for Adr unaffected by propranolol pretreatment was increased about 14 times by phentolamine pretreatment. The value for dopamine was also increased about 7 times with phentolamine but rather decreased with propranolol. On the other hand, the concentration-response curve of Isop was shifted to the right in a parallel fashion only by pretreatment with propranolol, giving a pA_2 value of 7.6 (slope 0.95, $n = 9$). In experiments to obtain the IC_{50} value, the influence of combined pretreatment with propranolol (3 μM) and phentolamine (1 μM) could not be examined, since the heights of the twitch responses were inconsistent in such conditions and thus the concentration-response relationship of catecholamines could not be estimated exactly. The twitch inhibition induced by dopamine (300 μM) was unaffected by pretreatment with haloperidol (0.1–1 μM, $n = 5$).

Effects of catecholamines on the submaximal contraction induced by acetylcholine

Exogenously applied ACh, above 3 nM, produced a contraction of the isolated muscularis mucosae of the guinea-pig oesophagus, in a concentration-dependent manner. The concentration producing 50% of the maximal contraction was 69 ± 5.6 nM ($n = 15$). The submaximal contraction induced by ACh (20 nM) and that produced by electrical stimulation (101.5 ± 6.7%, $n = 47$) were of similar magnitude. NA (1 μM), Adr (1 μM) and Isop (0.1 μM) inhibited the ACh (20 nM)-induced contraction by 84.6 ± 12.6% ($n = 14$), 75 ± 11.9% ($n = 14$) and 100% ($n = 10$), respectively. However, dopamine (300 μM) did not significantly modify the contraction induced by ACh ($n = 8$). The inhibitory actions of the catecholamines on the ACh-induced contractions were unaffected by pretreatment with phentolamine (1 μM). However, in the presence of propranolol (1 μM) NA (1 μM), Adr (1 μM) and dopamine (300 μM) potentiated the ACh-induced contraction some 2 to 2.5 times, and antagonized the inhibitory action of Isop (0.1 μM). Thesę potentiating actions of catecholamines were largely antagonized by phentolamine (1 μM).

Direct actions of catecholamines on the muscularis mucosae

In the presence of propranolol (3 μM), NA and Adr, above 0.1 μM, produced a weak contraction of the muscularis mucosae. The contractile response was slow in onset, reached the maximum 120 s after addition of the drug; after about 6 min the original muscle tone was restored. When the contractile response had reached the maximum, further addition of a higher concentration of NA or Adr depressed the preceding one. As shown in Figure 3, both concentration-response curves to NA and Adr were bell-shaped. The maximum responses to NA (30 μM) or Adr (30 μM) were only about 6 or 14%, respectively, of the maximum contraction produced by carbachol (3 μM), and were abolished by phentolamine (3 μM, $n = 14$). Dopamine also produced contraction of the muscularis mucosae at concentrations higher than 100 μM, which was less than 5% of the carbachol-induced maximum contraction. These contractile responses were unaffected by atropine (0.1 μM). Isop did not show any contractile response at the concentrations examined (0.1–300 μM).

On the other hand, all catecholamines, in the presence of phentolamine (10 μM), relaxed the muscularis mucosae which had been contracted by carbachol (3 μM), in a concentration-dependent manner (Figure 4). The maximum relaxation produced by Isop, NA and Adr reached about 90–95% of that induced by papaverine (30 μM), whereas that to dopamine was only 40% of the maximum even at a concentration of 300 μM. The concentrations (nM) of

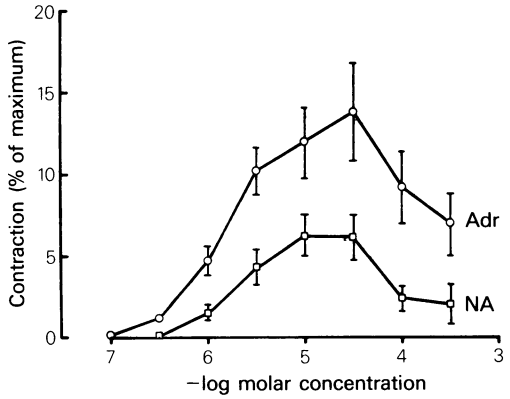

Figure 3 Concentration-response curves for the contractile responses to adrenaline and noradrenaline of the isolated muscularis mucosae of the guinea-pig oesophagus in the presence of propranolol (3 μM): adrenaline (Adr, ○, $n=16$); noradrenaline (NA, □, $n=20$). Adr and NA were applied to the tissue by the single dose technique, in which each concentration of Adr or NA was applied at random with a 30 min interval between doses. At the concentration ranges from 0.1 to 30 μM, Adr and NA produced a concentration-dependent contraction but at concentrations higher than 100 μM the contractile responses were smaller. The maximum contractions produced by 30 μM of Adr and of NA were only about 14% and 6% of that produced by carbachol (3 μM), respectively. Each point represents the mean response; vertical lines show s.e.mean. Ordinate scale shows % of the maximum contraction of this tissue produced by carbachol (3 μM).

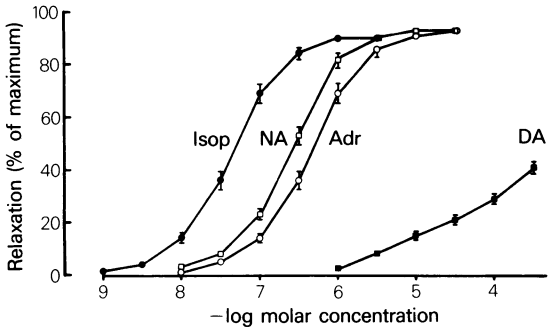

Figure 4 Cumulative concentration-response curves of catecholamines in relaxing the isolated muscularis mucosae of the guinea-pig oesophagus which was contracted maximally with carbachol (3 μM) in the presence of phentolamine (10 μM): isoprenaline (Isop, ●, $n=21$); noradrenaline (NA, □, $n=19$); adrenaline (Adr, ○, $n=19$); dopamine (DA, ●, $n=19$). All catecholamines produced a concentration-dependent relaxation in the carbachol-contracted muscularis mucosae; the order of potency was Isop > NA > Adr > DA. Each point represents the mean response; vertical lines show s.e.mean. Ordinate scale shows % of the maximum relaxation produced by papaverine (30 μM). This concentration of papaverine completely abolished the carbachol-induced tone.

Isop, NA and Adr required to relax the maximum contraction produced by carbachol by 50% (EC_{50}, mean ± s.e.mean) were 45.7 ± 4.1 ($n=20$), 269.2 ± 23.8 ($n=19$) and 478.6 ± 43.1 ($n=19$), respectively. The inhibitory responses produced by the catecholamines were competitively antagonized by propranolol. The pA_2 values for propranolol with Isop, NA and Adr were 7.78 (slope 1.23, $n=10$), 8.10 (slope 0.99, $n=10$) and 8.25 (slope 1.23, $n=10$), respectively. The dopamine (300 μM)-induced relaxation was completely antagonized by propranolol (1 μM, $n=6$), but not by haloperidol (0.1–10 μM, $n=5$).

Discussion

In the present experiments, the electrically-induced twitch contractions of the submucous plexus-longitudinal muscularis mucosae preparation of the guinea-pig oesophagus were inhibited by catecholamines, in a concentration-dependent and reversible manner, and above 1 μM, Isop, Adr and NA, but not dopamine, completely abolished the twitch. The order of potency was Isop > Adr > NA > dopamine. The inhibitory actions of NA and Adr were only partly reversed by phentolamine or propranolol separately, but abolished by both antagonists together. The inhibitory actions of the catecholamines involve both α and β-adrenoceptors. As suggested from the changes in the IC_{50} values induced by phentolamine and propranolol (Table 1), Adr preferentially activates α- rather than β-adrenoceptors, whereas the converse is ture for NA, since the value for Adr was increased more by phentolamine than propranolol, whereas that for NA was significantly increased only by propranolol. On the other hand, the inhibitory actions of dopamine and Isop seem to be mediated solely by α- and β-adrenoceptors, respectively, since phentolamine and propranolol alone, respectively, were completely effective (Table 1). The α-adrenoceptor-mediated inhibition seems to be due to a prejunctional reduction of ACh release from cholinergic nerves in the submucous plexus. Thus, in the presence of propranolol, NA, Adr and dopamine did not depress but rather enhanced the contraction induced by ACh (20 nM). However, the β-adrenoceptor-mediated inhibition may be postjunctional, because, in the presence of phentolamine, NA, Adr and Isop depressed the ACh-induced contraction to a similar extent as the electrically-induced twitch. Indeed all the catecholamines, in the presence of phentolamine,

relaxed the longitudinal muscularis mucosae contracted by carbachol (3 μM). The order of potency was Isop > NA > Adr > dopamine and their effects were competitively antagonized by propranolol. In the presence of propranolol, NA, Adr and dopamine, but not Isop, conversely produced a weak contraction of the muscularis mucosae. Since this effect was abolished by phentolamine, but not by atropine, the additional presence of excitatory postjunctional α-adrenoceptors is suggested (see also Walder, 1953; Bailey, 1965; Kamikawa & Shimo, 1979). In spite of evidence (Tansy, Martin, Landin & Kendall, 1979) to the contrary, no excitatory β-receptors were found while the absence of dopamine receptors was confirmed (Cox & Ennis, 1980). The submucous plexus-longitudinal muscularis mucosae of the guinea-pig oesophagus has three types of adrenoceptors, pre- and postjunctional α-adrenoceptors and postjunctional β-adrenoceptors; cholinergic transmission is inhibited by catecholamines acting at prejunctional α- and postjunctional β-adrenoceptors.

The difference in modulation by catecholamines of cholinergic neurotransmission between the submucous plexus preparation used here and the myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum is the strong involvement of postjunctional β-adrenoceptors. In longitudinal smooth muscles of the guinea-pig ileum, it is well known that catecholamines produce a β-adrenoceptor-mediated relaxation but the twitch inhibitory actions of NA and Adr are almost completely antagonized by α-blockers, and only partly by β-blockers, and the Isop-induced maximum twitch inhibition was only about 40% (Munro, 1951; Wilson, 1964; Kosterlitz *et al.*, 1970; Wikberg, 1977; 1978; Bauer, 1981). Furthermore, Isop 0.16 μM inhibited the submaximal contraction to ACh by about 84% (Kosterlitz, *et al.*, 1970), and Isop 4 μM relaxed by about 60% the contraction induced by carbachol (3 μM) (Wikberg, 1977). These values are lower than those observed in the present preparation; the difference in β-receptor potency in these two preparations may be due to differences in efficacy (or intrinsic activity) of the agonist-β-adrenoceptor complex for the production of inhibitory responses. The difference could also be explained by there being fewer β-adrenoceptors in the longitudinal muscle of the guinea-pig ileum. Another possibility is that the inhibitory β-adrenoceptors and the excitatory muscarinic receptors in the oesophageal muscularis mucosae may be more closely linked to each other than those in the ileum. Previously, Wilson (1964) found that catecholamines showed a more pronounced inhibition of histamine than of methacholine in the guinea-pig ileum. In preliminary experiments, however, we found that catecholamine-induced relaxations were of similar magnitude irrespective of whether the preparation had been contracted by carbachol or by histamine (Kamikawa & Shimo, 1979).

The physiological significance of modulation by catecholamines of the submucous plexus-longitudinal muscularis mucosae is not clearly established from the present results. Previously, Hirst and his colleagues found that an appreciable proportion of the neurones in the submucous plexus of the guinea-pig small intestine received a single inhibitory synaptic input (Hirst & McKirdy, 1975) and that iontophoretic application of NA and dopamine could mimic the inhibitory synaptic potentials (Hirst & Silinsky, 1975). Nozdrachev & Vataev (1981) also observed a similar inhibitory adrenergic input in the submucosal plexus of the cat small intestine. However, adrenergic modulation in the present tissue may be primarily driven by circulating catecholamines (Adr and NA) released from adrenal medulla, for the following reasons: (a) adrenergic innervation was very sparse in the guinea-pig oesophagus and any adrenergic nerve fibres did not surround the ganglion cells of the submucous plexus (Terayama & Soda, 1967; Kamikawa & Shimo, 1979; Cox & Ennis, 1980); (b) Adr was more potent (about 3 times) than NA in inhibiting the twitch (Figure 2 and Table 1); (c) the twitch inhibition by NA seemed to be mediated by postjunctional inhibitory β-adrenoceptors rather than prejunctional inhibitory α-adrenoceptors (Figure 1 and Table 1).

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