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SPECIAL REPORT

Noradrenaline-induced relaxation of rat oesophageal muscularis mucosae: mediation solely by innervated β_3-adrenoceptors

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We investigated the effects of cocaine and corticosterone on the noradrenaline-induced relaxation of rat oesophageal smooth muscle in the absence and presence of the selective β_2-antagonist, ICI 118,551. It was found that the concentration-response curve (CRC) of noradrenaline was not shifted by ICI 118,551 at 1 μM, whereas a clear shift to the right was observed at 100 μM of the antagonist. In the presence of corticosterone (10 μM), CRCs were clearly shifted to the left; with cocaine (10 μM) additionally present, a further leftward shift was observed, indicating the involvement of both neuronal and extraneuronal uptake sites. It was concluded that the relaxation of rat oesophageal muscularis mucosae by noradrenaline is solely mediated by β_3-adrenoceptors which are sympathetically innervated.

Keywords: β_3-Adrenoceptors; rat oesophagus; sympathetic innervation; cocaine; corticosterone

Introduction Pharmacological studies of oesophageal muscularis mucosae in guinea-pig (Kamikawa & Shimo, 1979), rat (Bieger & Triggle, 1985), dog, cat and opossum (Christensen & Percy, 1984), have shown that the smooth muscle is chiefly innervated by excitatory motor cholinergic nerves and, at least in the rat, only sparsely supplied with adrenergic fibres. Recently, we reported the existence of a major β_3-adrenoceptor population which, beside β_2-adrenoceptors, mediate relaxation in rat oesophagus; no evidence for any role of a β_1-adrenoceptor was found (De Boer *et al.*, 1993). It is not known whether the β_2- and/or β_3-adrenoceptors receive an adrenergic innervation or not.

Using electrical stimulation of para-arterial sympathetic neurones, Taneja & Clarke (1992) demonstrated that both the β_1- and the β_3-adrenoceptors in guinea-pig ileum are innervated. A different approach was made by Zaagsma *et al.* (1983), investigating β_1- and β_2-adrenoceptor-mediated relaxation of guinea-pig airways. They found that the contribution of β_1-adrenoceptors decreased from trachea to bronchi, which was in the same direction as the decrease in density of adrenergic innervation (O'Donnell *et al.*, 1978). Interestingly, these differences in innervation were also reflected by the effects of both neuronal and extraneuronal uptake inhibition on the noradrenaline- and isoprenaline-induced relaxation, respectively, which were pronounced in tracheal, but completely absent in bronchial preparations. A quantitative relationship was even indicated between the densities of noradrenergic innervation and postsynaptic β_1-adrenoceptors (Zaagsma *et al.*, 1987). This was supported by the observations that the catecholamine-induced relaxation of human central and peripheral airway smooth muscle, which are virtually devoid of noradrenergic nerves (Partanen *et al.*, 1982) is solely mediated by β_2-adrenoceptors and that no effects of neuronal and extraneuronal uptake inhibition occurred (Zaagsma *et al.*, 1983).

Since it is not known whether the β-adrenoceptor populations in rat oesophagus are innervated, we have studied the effects of neuronal and extraneuronal uptake inhibition by cocaine and corticosterone, respectively, on the (−)-noradrenaline-induced relaxation.

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Methods Oesophageal smooth muscle strips from male Wistar rats (260–300 g) were prepared as described previously (De Boer *et al.*, 1993). After dissection of the outer striated muscles, longitudinal strips of the muscularis mucosae were prepared and mounted in 20 ml water-jacketed organ baths filled with Krebs-Henseleit buffer solution, gassed with 95% O_2/5% CO_2, pH 7.4, 37°C, for isotonic recording under 0.2 g load. After equilibration for a period of at least 30 min, tissues showed neither resting tone nor spontaneous activity throughout the experiments.

After two methacholine concentration-response curves (CRCs) had been obtained, the preparations were contracted with methacholine (1 μM), which induced approximately 50% of the maximal contraction. When appropriate, corticosterone (10 μM), cocaine (10 μM), pargyline (10 μM) or ICI 118,551 (1 or 100 μM) were added 30 min before addition of noradrenaline. All CRCs were normalized and data are presented as means ± s.e.mean of (n) determinations. Statistical analyses were performed using Student's two-tailed t test ($\alpha < 0.05$).

The following drugs were used: (−)-noradrenaline hydrochloride and pargyline hydrochloride, purchased from Sigma (St. Louis, U.S.A.), corticosterone from Organon (Oss, The Netherlands) and cocaine hydrochloride from Brocacef (Maarssen, The Netherlands). ICI 118,551 (erythro-1-(7-methylindan-4-yloxy)-3-(isopropylamine)-butan-2-ol) was a kind gift from Zeneca (Macclesfield, U.K.). All buffer salts were from Merck (Amsterdam, The Netherlands).

Results (−)-Noradrenaline induced a concentration-dependent relaxation of oesophageal smooth muscle ($pD_2 = 5.72 \pm 0.09$ (6)), which was potentiated after inhibition of extraneuronal uptake by corticosterone ($pD_2 = 6.16 \pm 0.04$ (6)). With cocaine additionally present to block neuronal uptake as well, the CRC was further shifted to the left by 0.62 log unit ($pD_2 = 6.78 \pm 0.05$ (6), Figure 1a). All shifts were statistically significant ($P < 0.01$). Inhibition of monoamine oxidase (MAO) by pargyline only marginally increased the (−)-noradrenaline-induced relaxations (Table 1).

In the presence of the selective β_2-antagonist, ICI 118,551, at a concentration (1 μM) sufficient to occupy virtually all of the β_2-adrenoceptors, the CRC of (−)-noradrenaline was essentially identical to that of control ($pD_2 = 5.75 \pm 0.05$ (6), Figure 1b). With corticosterone and cocaine additionally

Table 1 Influence of pargyline, corticosterone and cocaine on the concentration response curves of (−)-noradrenaline expressed as pD_2-values from 4 to 6 experiments

			+ICI 118,551 (1 μM)	
	pD_2	ΔpD_2*	pD_2	ΔpD_2*
Control	5.72 ± 0.09		5.75 ± 0.05	
+ Pargyline	5.96 ± 0.12	0.24 ± 0.08^{NS}		
+ Corticosterone	6.16 ± 0.04	0.44 ± 0.10	5.96 ± 0.04	0.21 ± 0.06
+ Corticosterone + cocaine	6.78 ± 0.05	1.06 ± 0.13	6.65 ± 0.05	0.90 ± 0.04

*$\Delta pD_2 = pD_2$ (with pargyline or corticosterone ± cocaine) − pD_2 (control).
NS: not significant, $P > 0.05$.

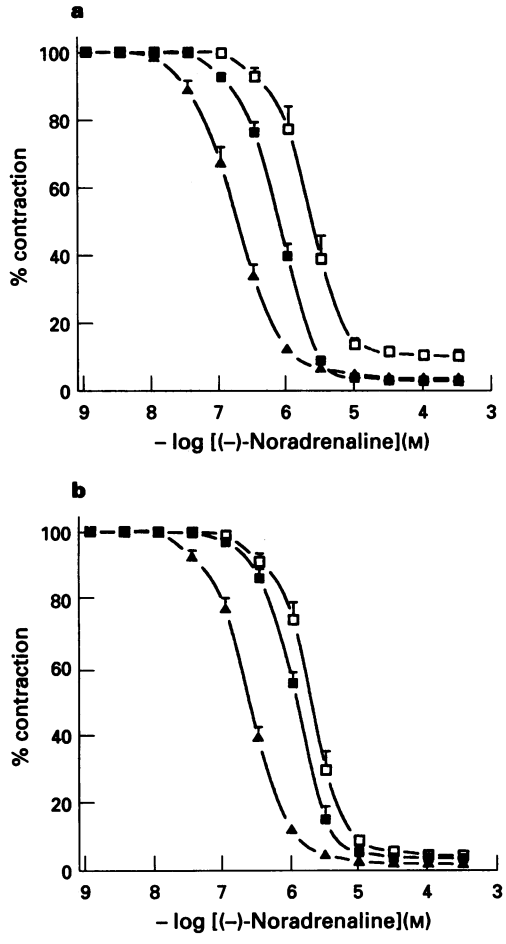

Figure 1 Potentiation of (−)-noradrenaline-induced relaxation in the absence (a) and presence of 1 μM ICI 118,551 (b). Control (□), corticosterone (10 μM, ■), corticosterone (10 μM) + cocaine (10 μM, ▲). Shown are the means of four to six experiments, each performed in duplicate.

present, a pronounced and significant leftward shift of the CRC to (−)-noradrenaline was again observed. While this shift was similar to and not significantly different ($P > 0.05$) from its counterpart without ICI 118,551, the shift produced by corticosterone alone (0.21 log unit) was significantly smaller ($P < 0.01$) (Table 1). At the high concentration (100 μM) of ICI 118,551, the CRC to (−)-noradrenaline was shifted to the right, yielding a pK_b value of 5.26 ± 0.13 (5).

Discussion The present study revealed two important features regarding β_3-adrenoceptor-mediated relaxation of rat oesophageal muscularis mucosae. First, (−)-noradrenaline-induced relaxations were unaffected by 1 μM of the β_2-antagonist, ICI 118,551, indicating that β_2-adrenoceptors are not involved. The pK_b value of 5.26, calculated from the rightward shift at the high concentration (100 μM) of ICI 118,551 is in full agreement with the reported pA_2-values of this antagonist for the β_3-adrenoceptor in rat oesophagus (De Boer *et al.*, 1993). Since in the latter study no evidence for the presence of β_1-adrenoceptors was found, this is, to our knowledge, the first demonstration that in rat oesophagus (−)-noradrenaline acts solely through β_3-adrenoceptors.

In addition, with neuronal and extraneuronal uptake sites blocked with cocaine and corticosterone, respectively, the CRC to (−)-noradrenaline is shifted to the left by approximately one log unit. This value is identical to the leftward shift (0.92 log unit) by corticosterone and cocaine of the CRC to (−)-noradrenaline in guinea-pig trachea (Zaagsma *et al.*, 1983) which is innervated by sympathetic nerves (O'Donnell *et al.*, 1978). Thus, the clear potentiating effects of neuronal and extraneuronal uptake inhibition agree with the presence of a noradrenergic innervation of rat oesophageal smooth muscle.

The potentiation of the noradrenaline-induced relaxation with corticosterone alone in the presence of ICI 118,551 (0.21 log unit) was slightly less than without the β_2-antagonist (0.44 log unit; $P < 0.01$), for which we have no explanation. However, the potentiating effects of cocaine in the presence of corticosterone (0.62 and 0.69 log unit in the absence and presence of ICI 118,551), are not significantly different and are well between 0.85 for, densely innervated, upper tracheal rings and 0.56 log units for, less innervated, lower tracheal rings previously reported (Zaagsma *et al.*, 1987). In addition, the experiments with pargyline show that the observed effects of cocaine cannot be explained by mucosal MAO-inhibition.

In conclusion, we have shown that in rat oesophageal muscularis mucosae (*i*) (−)-noradrenaline behaves as a full agonist acting entirely through β_3-adrenoceptors and (*ii*) that these receptors indeed receive a noradrenergic innervation, thereby further supporting the physiological role of the β_3-adrenoceptor.

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