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Relaxation of cat trachea by β-adrenoceptor agonists can be mediated by both β_1- and β_2-adrenoceptors and potentiated by inhibitors of extraneuronal uptake

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1 Responses (relaxation) to the β-adrenoceptor agonists, isoprenaline, fenoterol or noradrenaline, were obtained on cat tracheal preparations contracted with a submaximal concentration of carbachol (0.5 μM).

2 The relative potencies of isoprenaline: fenoterol: noradrenaline were 100:8.1:10.7. From this, it was concluded that responses were mediated predominantly by β_1-adrenoceptors but that a minor population of β_2-adrenoceptors might also be involved.

3 Schild plots for the selective antagonists atenolol (β_1-selective) or ICI 118,551 (β_2-selective) were in different locations, i.e. were separated, depending on whether the antagonist was antagonizing noradrenaline or fenoterol. This supported the conclusion that β_2- as well as β_1-adrenoceptors were involved in mediating the response. In this respect, cat trachea resembles cat atria (rate responses).

4 In the presence of atenolol the concentration-response curves to fenoterol became biphasic. This was interpreted as indicating that the β_2-adrenoceptors were too few in number to elicit a maximum tissue response.

5 Responses to isoprenaline of cat trachea were potentiated by the extraneuronal uptake inhibitor drugs, corticosterone and metanephrine. This indicated that extraneuronal uptake could modulate β-adrenoceptor-mediated responses (relaxation) of cat trachea.

6 Cat trachea resembles guinea-pig trachea in that (i) the β-adrenoceptor population mediating relaxation is mixed ($\beta_1 + \beta_2$) and (ii) responses to isoprenaline are modulated by its extraneuronal uptake. However, cat trachea differs from guinea-pig trachea in that the predominant β-adrenoceptor sub-type is β_1 not β_2.

Introduction

In cat trachea, the β-adrenoceptors mediating relaxation were classified by Lulich, Mitchell & Sparrow (1976) as β_1-adrenoceptors. Thus they appeared to be different from the β-adrenoceptors mediating responses in guinea-pig trachea, which were originally said to be β_2-adrenoceptors (Farmer & Levy, 1970). More recently, it has been possible to demonstrate that β_1 as well as β_2-adrenoceptors can mediate relaxation of guinea-pig trachea (Furchgott, 1976; O'Donnell & Wanstall, 1979a). Therefore it was of interest to determine whether tracheal relaxation in cat could also be mediated by both sub-types of β-adrenoceptor.

Because the β-adrenoceptor sub-type predominantly responsible for tracheal relaxation was apparently different in guinea-pig and cat, it was also of interest to determine whether the influence of extraneuronal uptake on β-adrenoceptor-mediated tracheal relaxation was also different in the two species. This question was relevant to the hypothesis that extraneuronal uptake may be more closely associated with β_2-adrenoceptors than β_1-adrenoceptors (Bryan, Cole, O'Donnell & Wanstall, 1981). An extraneuronal uptake of catecholamines has been demonstrated in the trachealis smooth muscle cells from both species, using a fluorescence histochemical technique (Anning, Bryan & O'Donnell, 1978) and responses to isoprenaline on guinea-pig trachea are known to be potentiated when this uptake is inhibited by extraneuronal uptake inhibitor drugs (O'Donnell & Wanstall, 1976). Experiments have now been carried out to determine whether

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potentiation of isoprenaline by extraneuronal uptake inhibitor drugs can be demonstrated on cat trachea. A preliminary report of some of these data formed part of a communication to the British Pharmacological Society (Cole, O'Donnell & Wanstall, 1982).

Methods

Cat tracheal preparations

Cats of either sex, weighing 1.5 to 3.5 kg, were pretreated with reserpine (0.1 mg/kg i.p. 18 h previously). They were anaesthetized with ether and then exsanguinated. The trachea was removed and single ring preparations (up to 4 preparations from each cat) were set up in Krebs solution at 37°C and gassed with 95% O_2 and 5% CO_2. In some experiments the whole tracheal ring was mounted, at a resting tension of 1.5 g, around two horizontal stainless steel pins (one fixed and one linked to a Statham Universal transducer, UC3 + UL5) and changes in isometric tension recorded. In other experiments, most of the cartilage was removed from the ring and the remaining smooth muscle preparation was set up, at a tension of 300 mg, so that changes in length could be recorded isotonically using a modified Statham 10B strain gauge. No quantitative differences were observed between these two types of preparation. Preparations taken from the region of the trachea nearest to the larynx are referred to as from the cervical trachea and those taken just before the trachea bifurcated into the bronchi are referred to as from thoracic trachea.

Preparations were contracted with a submaximal concentration (0.5 μM) of carbachol. Cumulative concentration-response (relaxation) curves were obtained to β-adrenoceptor agonist drugs and EC_{50} values (molar concentration of agonist producing 50% maximum response) interpolated. The potency of the agonist drugs was expressed as the negative log EC_{50}.

Schild plots for β-adrenoceptor antagonists on cat trachea

α-Adrenoceptors, neuronal uptake and extraneuronal uptake were blocked with phentolamine (10 μM), cocaine (10 μM) and metanephrine (50 μM) respectively (in contact with the tissue for 30 min before and then throughout the experiments). Concentration-response curves to either fenoterol (β_2-selective agonist) or noradrenaline (β_1-selective agonist) were obtained in the absence and presence of increasing concentrations of a β-adrenoceptor antagonist. The contact time for the antagonist drugs was 60 min. Except in the experiments with atenolol using fenoterol as agonist, when a different approach was used (see Results), values of concentration-ratio (CR) were calculated from the EC_{50} in the presence of the antagonist divided by the EC_{50} in the absence of antagonist (control). It was not necessary to correct these CR values since, in a separate series of experiments, it was shown that there was no significant change in sensitivity of the preparations with time and/or pre-exposure to the agonists. Plots of log (CR − 1) *vs.* log molar concentration of antagonist (log [B]) were obtained according to the method of Arunlakshana & Schild (1959). A linear least squares regression analysis (Snedecor & Cochran, 1967) was used to obtain the line of best fit through the combined data points from a number of cats, and this line is referred to as a Schild plot. None of the Schild plots had slopes which differed significantly from 1.0 (Table 2). Thus pA_2 values could be calculated for each concentration of antagonist used from the equation $pA_2 = \log (CR - 1) - \log [B]$ and a mean pA_2 value obtained for each cat. For each agonist-antagonist combination a mean pA_2 value ± s.e. was then calculated from a number of animals.

Effects of extraneuronal uptake inhibitors on isoprenaline-induced relaxation of cat trachea

α-Adrenoceptors and neuronal uptake were blocked with phentolamine (10 μM) and cocaine (10 μM) respectively. Concentration-response curves to isoprenaline were obtained in the absence and presence of metanephrine (50 μM) or of increasing concentrations (1, 10 and 50 μM) of corticosterone. The inhibitors were in contact with the tissue for 30 min before, and also during, the isoprenaline concentration-response curve. The corticosterone stock solution was made up in absolute ethanol. Therefore in the cortiscosterone experiments, a constant concentration of ethanol (17.2 mM) was maintained in the organ bath throughout the experiment by adding, when necessary, an appropriate amount of absolute ethanol to the Krebs solution in the organ bath before obtaining an isoprenaline concentration-response curve. Potentiation of isoprenaline, i.e. shift of the log concentration-response curve to a lower concentration range, was calculated from [neg log EC_{50} (inhibitor present) − neg log EC_{50} (control)]. In 4 of the 10 experiments with metanephrine the potentiation of isoprenaline was corrected to allow for antagonism of isoprenaline due to the weak β-adrenoceptor antagonist action of metanephrine (Kenakin, 1980). In order to do this, concentration-response curves were obtained for both fenoterol and isoprenaline (in random order), in the absence and presence of 50 μM metanephrine. Fenoterol is not a substrate for extraneuronal uptake and metanephrine antagonized responses to fenoterol, i.e. shifted the fenoterol concentration-response curves

to a higher concentration range. Thus corrected values for the potentiation of isoprenaline by metanephrine, due to inhibition of extraneuronal uptake, were obtained by adding the value for the antagonism of fenoterol (in log units) to the value for the potentiation of isoprenaline (in log units).

Cat right atrial preparations

Cat right atrial preparations were set up for recording atrial rate as described previously (O'Donnell & Wanstall, 1979b). Phenoxybenzamine (50 μM for 30 min followed by thorough wash out) was used to block α-adrenoceptors, neuronal uptake and extraneuronal uptake in these experiments. Schild plots and pA_2 values were obtained as described for cat tracheal preparations (see above).

Drugs and solutions

The following drugs were used: atenolol (I.C.I.); carbachol (Sigma); cocaine hydrochloride (Drug Houses of Australia); corticosterone (Sigma); fenoterol hydrobromide (Boehringer-Ingelheim); ICI 118,551 (erythro-DL-1(7-methylindan-4-yloxy)-3-*iso*propylaminobutan-2-ol, I.C.I.); (±)-isoprenaline sulphate (Sigma); (±)-metanephrine hydrochloride (Calbiochem); noradrenaline acid tartrate (Sigma); phenoxybenzamine hydrochloride (Smith, Kline & French); phentolamine methanesulphonate (Regitine ampoules, Ciba); reserpine (Serpasil ampoules, Ciba). Stock solutions (10 or 100 mM) of atenolol, fenoterol, ICI 118,551, isoprenaline, metanephrine and noradrenaline were made up in 0.01 M HCl and stock solutions of carbachol and cocaine in distilled water. Phenoxybenzamine was dissolved in absolute ethanol containing 0.01 M HCl to prepare a 100 mM stock solution. Corticosterone was dissolved in absolute ethanol to prepare a 50 mM stock solution. All dilutions of stock solutions were made in Krebs solution and kept on ice during the course of the experiment.

The composition of the Krebs solution was (mM): NaCl 114, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11.7, ascorbic acid 1.1.

Statistical analyses

Mean values are quoted together with their standard errors (s.e.). Comparisons have been made using either Student's *t* test or a paired *t* test.

Results

In preliminary experiments no significant difference was found between preparations from the cervical and thoracic trachea in their sensitivity to either fenoterol or noradrenaline. Mean negative log EC_{50} values for paired cervical and throracic preparations respectively were: fenoterol 6.42 ± 0.11, 6.20 ± 0.11 ($n = 5$); noradrenaline 6.70 ± 0.05, 6.60 ± 0.14 ($n = 4$). The thoracic trachea was selected for subsequent experiments because, in these preliminary experiments, concentration ratio (CR) values for the β-adrenoceptor antagonist drug, ICI 118,551, were less variable on thoracic than on cervical preparations.

The potencies and relative potencies of isoprenaline, noradrenaline and fenoterol on cat thoracic trachea are summarized in Table 1, together with data obtained on cat atria for comparison.

Schild plots for ICI 118,551 and atenolol on cat thoracic trachea

ICI 118,551 caused a parallel shift in the log concentration-response curves to both fenoterol and noradrenaline. Atenolol caused a parallel shift in the curves to noradrenaline, but the curves to fenoterol

Table 1 Potency values (mean neg log EC_{50} ± s.e.) and relative potency values of isoprenaline, fenoterol and noradrenaline on cat thoracic trachea and cat right atria

	Mean neg log EC_{50} ± s.e.		*Relative potency*[b]	
Agonist	*Trachea*	*Atria*	*Trachea*	*Atria*
Isoprenaline	7.38 ± 0.09 (6)[a]	8.82 ± 0.10 (12)	100	100
Fenoterol	6.29 ± 0.07 (10)	7.83 ± 0.07 (17)	8.1	10.2
Noradrenaline	6.71 ± 0.11 (8)	7.99 ± 0.06 (15)	10.7[c]	7.4[c]

[a] Number of animals

[b] Relative potency = 100 × antilog (neg log $EC_{50_{compound}}$ − neg log $EC_{50_{isoprenaline}}$)

[c] One half of the experimental value, to allow for the fact that (−)-noradrenaline was used whereas (±)-isoprenaline and (±)-fenoterol were used.

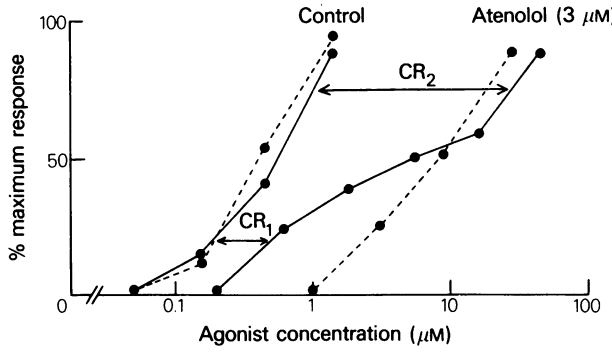

Figure 1 Concentration-response curves obtained on two adjacent thoracic rings from the same trachea. On one preparation fenoterol was the agonist (●——●) and on the other preparation noradrenaline was the agonist (●----●). Control curves and curves in the presence of 3 μM atenolol are shown. Atenolol caused a parallel shift of the noradrenaline concentration-response curve but a non-parallel shift of the fenoterol concentration-response curve. Two CR values for atenolol were obtained when fenoterol was the agonist (CR_1 and CR_2).

became biphasic in the presence of atenolol (Figure 1). Consequently, with fenoterol as agonist, two CR values for atenolol were determined as indicated in Figure 1, CR_1 corresponding to the first phase of the biphasic concentration-response curve, and CR_2 corresponding to the second phase. Two Schild plots were then obtained, based on the CR_1 and CR_2 values respectively. The Schild plots for ICI 118,551 and for atenolol are shown in Figure 2. The slopes of these Schild plots and the mean pA_2 values from these data are summarized in Table 2. For each of the antagonists the Schild plots were in different locations (Figure 2) and the pA_2 values were different (Table 2) depending on whether the agonist was noradrenaline or fenoterol. One of the two pA_2 values obtained for atenolol with fenoterol as agonist (i.e. the one based on CR_2 values) was the same as the pA_2 value for atenolol with noradrenaline as agonist (Table 2).

Schild plots for ICI 118,551 on cat atria

These data were obtained for comparison with those on cat trachea and the pA_2 values with fenoterol and noradrenaline as agonists together with the slopes of the Schild plots are summarized in Table 2.

Effects of extraneuronal uptake inhibitors on isoprenaline responses on cat thoracic trachea

Corticosterone caused potentiation of isoprenaline responses. Mean potentiation by 1 μM corticosterone was 0.15 ± 0.06 log units ($n = 5$), $P > 0.05$, paired t test; by 10 μM corticosterone was 0.41 ± 0.08 ($n = 5$), $0.01 > P > 0.001$; and by 50 μM corticosterone was 0.57 ± 0.14 ($n = 5$), $0.05 > P > 0.01$. Metanephrine (50 μM) also potentiated responses to isoprenaline but the potentiation (0.16 ± 0.06 log units, $n = 10$, $0.05 > P > 0.01$) was less than that produced by 10 or 50 μM corticosterone unless a correction was made for the weak β-adrenoceptor antagonist activity of metanephrine (see Methods). The potentiation of isoprenaline by metanephrine

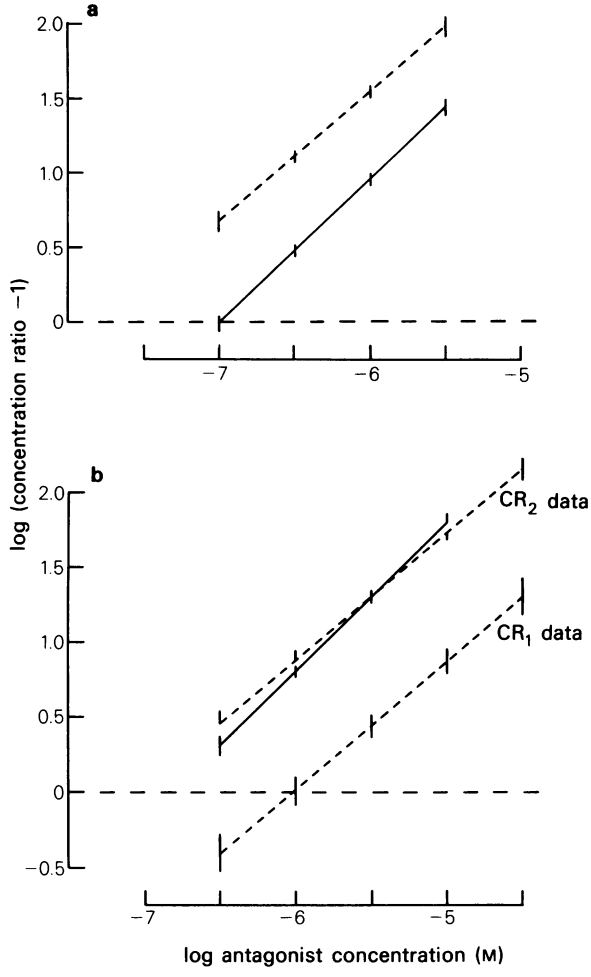

Figure 2 Schild plots for ICI 118,551 (a) and atenolol (b) on cat trachea using noradrenaline (——) or fenoterol (--------) as agonists. The plots are calculated regression lines of best fit through data points from a number of animals. The vertical bars represent the s.e. of the estimated values of log (CR − 1) at points corresponding to the antagonist concentrations used. The number of animals and number of data points are shown in Table 2.

Table 2 pA_2 values and slopes of Schild plots for ICI 118, 551 and atenolol on cat thoracic trachea and cat right atria obtained using fenoterol or noradrenaline as agonist

		Fenoterol as agonist	*Noradrenaline as agonist*
ICI 118,551	Trachea	7.55 ± 0.06 (5)*** [0.86 ± 0.08, 10][b]	6.96 ± 0.05 (4)[a] [0.96 ± 0.06, 12]
	Atria	7.37 ± 0.09 (6)** [1.12 ± 0.11, 9]	6.87 ± 0.08 (5) [0.99 ± 0.11, 9]
Atenolol	Trachea	5.94 ± 0.02 (5)***[c] [0.86 ± 0.11, 15] 6.80 ± 0.07 (5),[d] [0.85 ± 0.07, 15]	6.81 ± 0.02 (4) [0.99 ± 0.07, 12]
	Atria[e]	5.7 ± 0.12 (6)*** [0.90 ± 0.22, 10]	6.9 ± 0.11 (5) [0.92 ± 0.14, 9]

[a] Number of animals
[b] Slope of Schild plot ± s.e., number of data points.
[c] pA_2 obtained using CR_1 (Figure 1); [d] pA_2 obtained using CR_2 (Figure 1).
[e] Data from O'Donnell & Wanstall (1980a)
*** pA_2 value significantly different from that with noradrenaline as agonist $P < 0.001$ (Student's t test); ** pA_2 value significantly different from that with noradrenaline as agonist $0.01 > P > 0.001$ (Student's t test).

(50 μM) after making this correction was 0.60 ± 0.06 log units ($n = 4$), $0.01 > P > 0.001$.

Discussion

Under optimal experimental conditions (Furchgott, 1972), comparison of the potencies of noradrenaline and isoprenaline can indicate whether the predominant β-adrenoceptor sub-type mediating a response is β_1 or β_2. For example, noradrenaline has been shown to be about a tenth as potent as isoprenaline for responses mediated predominantly by β_1-adrenoceptors, *viz.* increase in atrial rate in guinea-pig and rat (β_1 only, O'Donnell & Wanstall, 1979b; Bryan *et al.*, 1981) and increase in atrial rate in cat ($\beta_1 > \beta_2$, O'Donnell & Wanstall, 1979b). In contrast, for tissue responses mediated predominantly by β_2-adrenoceptors, noradrenaline is approximately one hundredth as potent as isoprenaline, e.g. relaxation of guinea-pig trachea (O'Donnell & Wanstall, 1979a) and relaxation of rat pulmonary artery (O'Donnell & Wanstall, 1981a). In the present study on the relaxation of the thoracic trachea of cat, noradrenaline was one tenth as potent as isoprenaline. This indicated that β_1-adrenoceptors were the predominant receptor sub-type mediating the response. A preliminary indication of whether these β_1-adrenoceptors were the only receptor sub-type involved was then obtained by comparing the potencies of noradrenaline and fenoterol. For responses mediated solely by β_1-adrenoceptors (guinea-pig and rat atrial rate responses, O'Donnell & Wanstall, 1979b; Bryan *et al.*, 1981) noradrenaline is more potent than fenoterol. But in the present study noradrenaline was equipotent with fenoterol, suggesting that in cat trachea (as in cat atria) a minor population of β_2-adrenoceptors might also be involved in the response. This view was confirmed by results obtained with selective β-adrenoceptor antagonists. For both ICI 118,551 (β_2-selective, O'Donnell & Wanstall, 1980c) and atenolol (β_1-selective) the Schild plots obtained, using agonists with opposite selectivity for the β-adrenoceptor subtypes, *viz.* noradrenaline (β_1-selective) and fenoterol (β_2-selective), were not superimposed but were separated. This indicates a lack of homogeneity in a β-adrenoceptor population (O'Donnell & Wanstall, 1981b) and so confirmed the involvement of β_2- as well as β_1-adrenoceptors.

Although cat thoracic trachea was used for most of the present study, the preliminary observations on cervical trachea indicated that relaxation of these preparations also involved a β-adrenoceptor population that was not homogeneous. Noradrenaline and fenoterol were almost equipotent, and the results obtained with ICI 118,551, though more variable than those obtained on thoracic trachea, indicated that this drug did not antagonize noradrenaline and fenoterol responses equally.

In the experiments in which the antagonism of fenoterol by atenolol was studied, the log concentration-response curve to fenoterol became biphasic in the presence of atenolol. The first phase of the curve appeared to represent the action of fenoterol on β_2-adrenoceptors, since the pA_2 value for

atenolol corresponding to this phase of the response was 5.94, a value close to that determined for atenolol on β_2-adrenoceptors in other tissues (5.61, O'Donnell & Wanstall, 1979a). The maximum relaxation for this phase was between 30 and 60% of the isoprenaline maximum response. Since fenoterol is an agonist with a high intrinsic efficacy (O'Donnell & Wanstall, 1977), this suggests that the overall number of β_2-adrenoceptors in cat trachea is too small to mediate maximum relaxation of the tissue. As the concentration of fenoterol was increased, the second phase of the log concentration-response curve was seen and the pA_2 value of atenolol determined from this second phase was 6.80. This is close to the pA_2 value for atenolol on β_1-adrenoceptors in cat trachea (6.81, this study) and in other tissues (7.05, O'Donnell & Wanstall, 1979a). Stimulation of the β_1-adrenoceptors could mediate maximum tissue relaxation. Assuming this interpretation is correct, a slight steepening of the fenoterol log concentration-response curve could have been expected in the presence of the β_2-selective antagonist, ICI 118,551 but the present results did not detect this more subtle change.

Nevertheless, from these data, it appeared that the relaxation of cat trachea differed from the relaxation of guinea-pig trachea and rat pulmonary artery and from the increase in cat atrial rate in that, although these latter responses are all mediated by both the β-adrenoceptor sub-types, activation of either sub-type could apparently elicit a maximum tissue response (O'Donnell & Wanstall, 1979a,b; 1980a; 1981a). It is of interest that a biphasic log concentration-response curve to the β_2-selective agonist, procaterol, in the presence of the β_1-selective antagonist, pamatolol, has recently been described for the inotropic response in cat papillary muscle (Hedberg & Mattson, 1981).

It was also noted in the present study, which used carbachol-contracted tracheal preparations from cats, that the slopes of the Schild plots for both of the selective β-adrenoceptor antagonists used (atenolol and ICI 118,551) were close to 1.0. This was in contrast to the findings on guinea-pig carbachol-contracted trachea when Schild plots for the same antagonists had slopes significantly less than 1.0 (O'Donnell & Wanstall, 1980b,c). The guinea-pig trachea results complied with a recent model, proposed by Furchgott (1981), in which he predicted that, for an agonist response involving both β-adrenoceptor sub-types, the Schild plot for a competitive antagonist with a selectivity for one of the sub-types would have a slope less than 1.0 over a certain part of the antagonist concentation range. The extent of the deviation of the slope from 1.0 and also the antagonist concentration-range over which the deviation occurred would depend not only on the differences in the affinities of both antagonist and agonist for the two receptor sub-types but also on the relative proportions of the two receptor sub-types. Thus the slopes close to 1.0 in cat trachea, as opposed to the slopes less than 1.0 in guinea-pig trachea, may

Table 3 Comparison of pA_2 values for atenolol and ICI 118,551 on cat trachea and atria (noradrenaline or fenoterol as agonists) with pA_2 values on β_1- and β_2-adrenoceptors in other tissues

		pA_2 values	
		Atenolol	*ICI 118,551*
'β_1'		7.05[a]; 7,28[b]; 7.37[c]; 6.78[d]; 6.83[e]	6.96[a]; 6.83[d]; 6.75[e]; 6.78[f]
Cat trachea	(agonist, noradrenaline)	6.81	6.96
Cat atria	(agonist, noradrenaline)	6.9	6.87
'β_2'		5.61[g]; 5.03[h]	8.69[g]; 9.16[h]; 8.78[i]
Cat trachea	(agonist, fenoterol)	5.94	7.55
Cat atria	(agonist, fenoterol)	5.7	7.37

[a] Guinea-pig atria (agonist noradrenaline); O'Donnell & Wanstall (1979a, 1980c)
[b] Rat atria (agonist fenoterol); Bryan *et al.* (1981)
[c] Rat atria (agonist noradrenaline); Bryan *et al.* (1981)
[d] Guinea-pig atria (agonist fenoterol); O'Donnell & Wanstall (1979a, 1980c)
[e] Guinea-pig atria (agonist isoprenaline); O'Donnell & Wanstall (1979a, 1981b)
[f] Guinea-pig atria (agonist adrenaline); O'Donnell & Wanstall (1981b)
[g] Guinea-pig trachea (agonist fenoterol); O'Donnell & Wanstall (1979a, 1980c)
[h] Rat pulmonary artery (agonist fenoterol); O'Donnell & Wanstall (1981a)
[i] Guinea-pig trachea (agonist salbutamol); O'Donnell & Wanstall (1981b)

support the conclusion that the minor receptor population (β_2) in cat trachea is small compared with the minor receptor population (β_1) in guinea-pig trachea.

In light of the mixed population of β-adrenoceptors in both cat trachea and cat atria, it is of interest to compare the pA_2 values for the selective antagonists obtained on these tissues with their pA_2 values already proposed on β_1- and β_2-adrenoceptors. Table 3 summarizes the pA_2 values which could be considered to most closely represent the pA_2 values of atenolol and ICI 118,551 on β_1- and β_2-adrenoceptors respectively. In selecting these data the conclusion of O'Donnell & Wanstall (1979a) has been adopted *viz.* that the best estimate of the pA_2 on β_1-adrenoceptors is that obtained either on a tissue containing only β_1-adrenoceptors, using any agonist, or on a tissue containing predominantly β_1-adrenoceptors and using a β_1-selective agonist. Conversely, the best estimate of the pA_2 on β_2-adrenoceptors is obtained on a tissue containing only β_2-adrenoceptors, using any agonist (no data yet available), or on a tissue containing predominantly β_2-adrenoceptors using a β_2-selective agonist. The pA_2 values for both atenolol and ICI 118,551 on cat trachea and atria with noradrenaline as agonist agreed well with the 'β_1' pA_2 values for these drugs (Table 3). Likewise the pA_2 values for atenolol with fenoterol as agonist were in fairly good agreement with the 'β_2' pA_2 values for this drug. However, the values for ICI 118,551 with fenoterol as agonist were at least one log unit lower than its 'β_2' pA_2 value (Table 3). Hence it appears that on a mixed receptor tissue in which β_1-adrenoceptors predominate, the pA_2 value for a β_2-selective antagonist, even when obtained using a β_2-selective agonist, is an underestimate of its 'β_2' pA_2 value.

In the present study both corticosterone and metanephrine potentiated responses of cat trachea to isoprenaline. This observation appears to contradict the findings of Lulich *et al.* (1976) who failed to demonstrate potentiation of adrenaline by normetanephrine. In the experiments of Lulich *et al.* (1976) neuronal uptake was not blocked, even though adrenaline was the agonist used, and allowance was not made for possible β-adrenoceptor blocking activity of normetanephrine. Both these factors may have made potentiation, resulting from inhibition of extraneuronal uptake, difficult to detect. The potentiation data in the present study confirmed that in cat trachea the extraneuronal uptake process, observed in fluorescence histochemistry experiments (Anning *et al.*, 1978), could modulate β-adrenoceptor-mediated responses, i.e. was functionally effective. Potentiation of isoprenaline responses by extraneuronal uptake inhibitors has now been demonstrated in tissues in which β_2-adrenoceptors are involved in the response, whether the β_2-adrenoceptors are the predominant sub-type present (guinea-pig trachea, O'Donnell & Wanstall, 1976) or are in the minority (cat trachea, this study; cat heart, Kaumann, 1972; Goldie, 1976). However, potentiation has yet to be demonstrated in tissues in which it has been established that β_2-adrenoceptors are not involved at all in β-adrenoceptor-mediated responses.

In conclusion, cat trachea resembles guinea-pig trachea in that (a) responses are mediated by a mixed population of both β_1- and β_2-adrenoceptors, and (b) β-adrenoceptor-mediated responses are potentiated by extraneuronal uptake inhibitor drugs. However, cat trachea differs from guinea-pig trachea in that the predominant β-adrenoceptor sub-type involved in the relaxation response is β_1. Since β_1-adrenoceptors are more sensitive than β_2-adrenoceptors to noradrenaline, one could speculate that this observation may be related to the denser adrenergic innervation in cat trachea (compared with guinea-pig) and/or the fact that the adrenal medulla of cat, but not guinea-pig, contains noradrenaline as well as adrenaline (Shepherd & West, 1951).

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