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Stereoselectivity of extraneuronal uptake of catecholamines in guinea-pig trachealis smooth muscle cells

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1 The extraneuronal uptake of the (−)- and (+)-isomers of three catecholamines, isoprenaline, adrenaline and noradrenaline, were compared in guinea-pig trachealis smooth muscle cells, by a fluorescence microphotometric method.

2 Preliminary experiments showed that the initial rates of uptake of the (−)-isomers were greater than those of the (+)-isomers in tissues incubated in 25 μM adrenaline or noradrenaline or 50 μM isoprenaline.

3 More detailed experiments showed that the K_m values of the (+)-isomers of the amines for extraneuronal uptake were not significantly different from each other, but the K_m value of (−)-isoprenaline < (−)-adrenaline < (−)-noradrenaline. Thus, the order of the ratios of the K_m values for the (+):(−)-isomers was isoprenaline > adrenaline > noradrenaline.

4 The results showed that there is stereoselectivity of the extraneuronal uptake of catecholamines, but it is greatest for isoprenaline (4.9 fold), less for adrenaline (2.5 fold) and almost negligible (1·6 fold) for noradrenaline.

Introduction

It has generally been accepted that the extraneuronal uptake of catecholamines in tissues cannot distinguish between the (−)- and (+)-isomers of these amines, i.e. is not stereoselective (see Gillespie, 1976). This conclusion was based on the results of Iversen (1965) who found that there was no difference between the accumulation of (−)- and (+)-noradrenaline or of (−)- and (±)-adrenaline by Uptake$_2$ (extraneuronal uptake) in the rat heart. In the present study, the affinities of the (−)- and (+)-isomers of isoprenaline, as well as of adrenaline and noradrenaline, for extraneuronal uptake in guinea-pig trachealis smooth muscle cells have been obtained, by use of a fluorescence microphotometric method. Comparison of these values indicates that extraneuronal uptake can show stereoselectivity.

A preliminary account of this work was presented to the Australasian Society of Clinical and Experimental Pharmacologists (Bryan & O'Donnell, 1984).

Methods

Adult, female guinea-pigs (360–590 g) were pretreated with 6-hydroxydopamine 50 mg kg^{-1}, intravenously, 24 h before the experiment, to achieve chemical sympathectomy (O'Donnell & Saar, 1974). The guinea-pigs were killed by a blow on the head, and the trachea from each animal was cut into the appropriate number of intact rings, approximately two cartilages thick.

The experiments were carried out as described by Bryan & O'Donnell (1980a). The Krebs solution used in the experiments contained (in mM) NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11.7 and ascorbic acid 1.14, and was aerated with 5% CO_2 in O_2. In experiments with adrenaline or noradrenaline, monoamine oxidase was inhibited by pargyline 100 μM (Bryan & O'Donnell, 1980a), and, in all experiments, catechol-*O*-methyltransferase was inhibited by U-0521 100 μM (Bryan & O'Donnell, 1980a). When an extraneuronal uptake inhibitor was required (see below), corticosterone 100 μM was included in the Krebs solution throughout the experiment. Tracheal rings were incubated in (−)- or (+)-isoprenaline, adrenaline or noradrenaline, at the specified concentration (25–1200 μM), at 37°C for 5 min. This incubation time was selected, because the accumulation of the amines has been shown to occur at close to

a constant rate over this period (Bryan & O'Donnell, 1980b). Thus, measurements of the accumulation of amine in the cells reflected uptake into the cells, without any complications due to efflux. Extracellular and loosely bound catecholamine was then removed by washing the tissues in amine-free Krebs solution at 0–1°C for 30 min.

The tracheal rings were prepared for fluorescence histochemistry, and the accumulation of catecholamine in the trachealis smooth muscle cells was quantified by obtaining fluorescence intensity measurements in 7 μm sections with a Leitz MPV microphotometer (Bryan & O'Donnell, 1980a). Each fluorescence intensity value, expressed in arbitrary units (F), was corrected for background fluorescence measured in a corresponding control tissue which was from the same animal, but not exposed to a catecholamine. The resultant values are referred to as corrected fluorescence intensity values. Rates of uptake of the catecholamines (in $F\,min^{-1}$) were calculated by dividing the corrected fluorescence intensity values by the time of incubation in the amine (5 min).

Treatment of data

The initial rates of total uptake of catecholamines into the cells (measured in tissues incubated in the amine, in the absence of corticosterone) represented entry of the amine both by extraneuronal uptake, which could be inhibited by corticosterone, and by diffusion, which is resistant to corticosterone (Bryan *et al.*, 1984). Under experimental conditions where there was no significant entry of the amine by diffusion (see below), total uptake was assumed to represent extraneuronal uptake. Under conditions where there could be significant entry of the amines by diffusion, the initial rates of uptake of amine into the cells were measured both in the absence (total uptake) and in the presence of corticosterone 100 μM. The uptake measured in the presence of corticosterone (100 μM) represented diffusion of the amine *plus* any extraneuronal uptake which was not inhibited by this concentration of corticosterone. Corticosterone is a competitive inhibitor of extraneuronal uptake (Bryan & O'Donnell, 1981), and there was incomplete inhibition of extraneuronal uptake by corticosterone (100 μM) because high concentrations of the substrate amines were used in this study. Thus, this small amount of residual extraneuronal uptake was calculated, using equations for competitive inhibition (details in Bryan *et al.*, 1984), and the amine entry which still remained in the presence of corticosterone was taken to represent diffusion. The difference between total uptake and diffusion was the extraneuronal uptake of the amine into the cells.

For isoprenaline, data were obtained in the absence and presence of corticosterone (100 μM) at each concentration of isoprenaline ((−)-isomer: 25, 50, 100, 200,400 μM; (+)-isomer: 50, 100, 200, 400, 800 μM). These data provided initial rates of both extraneuronal uptake and diffusion at each concentration of isoprenaline.

For adrenaline and noradrenaline, data were obtained for 400 and 800 μM of the (−)-isomers, and 400, 800 and 1200 μM of the (+)-isomers in the presence of corticosterone (100 μM). These data allowed the initial rates of total uptake (measured in the absence of corticosterone) for these concentrations of adrenaline and noradrenaline to be corrected for diffusion. For amine concentrations lower than 400 μM, diffusion of adrenaline and noradrenaline into guinea-pig trachealis smooth muscle cells has been shown to be negligible (Bryan *et al.*, 1984). Thus, initial rates of total uptake were assumed to represent initial rates of extraneuronal uptake. It should be noted that the data for the total uptake of the (−)-isomers of adrenaline and noradrenaline were obtained from a previous study (Bryan & O'Donnell, 1980b).

Table 1 Initial rates of extraneuronal uptake of (−)- and (+)-isomers of isoprenaline, adrenaline and noradrenaline in guinea-pig trachealis smooth muscle cells.

Amine	*Concentration* (μM)	*Initial rate of extraneuronal uptake* ($F\,min^{-1}$) Mean ± s.e. (−)-isomer	(+)-isomer
Isoprenaline	50	52.6 ± 5.82	15.2 ± 1.82*
Adrenaline	25	36.5 ± 1.02	20.9 ± 0.55***
Noradrenaline	25[a]	42.8 ± 0.68	34.0 ± 1.86***

Data are from tissues from 4 guinea-pigs except for results marked [a] where 8 guinea-pigs were used.
Values for (+)-isomers which are significantly different from those for the corresponding (−)-isomers: * $0.05 > P > 0.01$; *** $P < 0.001$ (paired *t* test).

Statistics

Values for rates of uptake are expressed as arithmetic means ± s.e. Where appropriate, data were analyzed by linear least squares regression analysis (Snedecor & Cochran, 1980). K_m values were obtained by a method for least squares estimation of non-linear parameters (Marquardt, 1963), and are expressed as geometric means with 95% confidence limits. The significance of differences between values was assessed by Student's t test or paired t test as indicated, using absolute or log values depending on whether arithmetic or geometric means, respectively, had been calculated.

Drugs and solutions

The drugs used in the study were: (−)-adrenaline bitartrate (Sigma Chemical Co.); (+)-adrenaline bitartrate (Lilly Research Laboratories); corticosterone (Sigma); 3′,4′-dihydroxy-2-methylpropiophenone (U-0521; Upjohn); 6-hydroxydopamine hydrobromide (Sigma); (−)-isoprenaline bitartrate (Sigma); (+)-isoprenaline bitartrate (Sigma); (−)-noradrenaline bitartrate (Sigma); (+)-noradrenaline bitartrate (Sterling-Winthrop); pargyline hydrochloride (Sigma).

Stock solutions and their dilutions were prepared as described by Bryan *et al.* (1984).

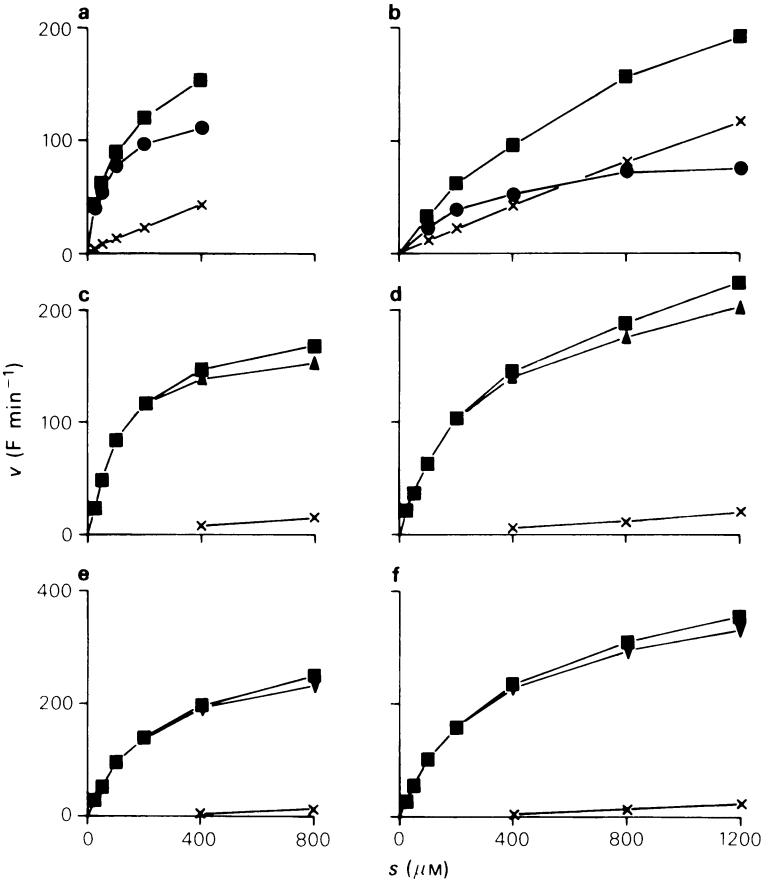

Figure 1 Kinetic analyses of the uptake of the (−)-isomers (left-hand graphs) and (+)-isomers (right-hand graphs) of isoprenaline (a,b), adrenaline (c,d) and noradrenaline (e,f) in guinea-pig trachealis smooth muscle cells. Mean initial rates of uptake (v, in F min^{-1}) are plotted against amine concentration (s, in μM) for total uptake (■), extraneuronal uptake (●, ▲, ▼) and diffusional entry (×). Standard errors were smaller than the size of the symbols. The data for total uptake of (−)-adrenaline (c) and (−)-noradrenaline (e) are from Bryan & O'Donnell (1980b) and are from tissues from 5 guinea-pigs; all other data are from tissues from 4 guinea-pigs. The maximum initial rates of extraneuronal uptake (in F min^{-1}) appeared to be greater for the (+)-isomers of adrenaline (d) and noradrenaline (f) than for the corresponding (−)-isomers (c,e), but these differences reflect slight variations in actual fluorescence intensity values in experiments carried out at different times, even though K_m values are highly reproducible.

Table 2 K_m values of (−)- and (+)-isomers of isoprenaline, adrenaline and noradrenaline for extraneuronal uptake in guinea-pig trachealis smooth muscle cells

Amine	K_m (μM) Mean (95% confidence limits) (−)-isomer	(+)-isomer
Isoprenaline	67.7(51.8; 88.7)†††	331(225; 486)***
Adrenaline	127(112; 144)[a]	313(262; 375)***
Noradrenaline	207(149; 288)[a],††	334(213; 524)

Data for each isomer are from tissues from 4 guinea-pigs, unless otherwise indicated.
[a] Values obtained from K_m values of 151 μM for (−)-adrenaline and 238 μM for (−)-noradrenaline (Bryan & O'Donnell, 1980b) by correction of data for diffusion, when the amine concentration was greater than 200 μM; data from 5 guinea-pigs.
Values for (+)-isomers which are significantly different from those for the corresponding (−)-isomers: *** $P < 0.001$ (Student's t test).
Values which are significantly different from those for the corresponding isomer of adrenaline: †† $0.01 > P > 0.001$; ††† $P < 0.001$ (Student's t test).

Results

The initial rates of extraneuronal uptake of the (−)- and (+)-isomers of isoprenaline, adrenaline and noradrenaline, in trachealis smooth muscle cells in tissues which had been incubated in 25 μM of either adrenaline or noradrenaline or in 50 μM isoprenaline, are summarised in Table 1. These results indicated a difference between the rates of uptake of the (−)- and (+)-isomers of each of the amines, i.e. the rate for the (−)-isomer was 3.5 fold, 1.7 fold and 1.3 fold greater than that for the (+)-isomer for isoprenaline, adrenaline and noradrenaline respectively.

More detailed experiments in which the initial rates (v) of total uptake, extraneuronal uptake and diffusional entry for the various isomers were obtained, are illustrated in Figure 1. The amine concentrations (s) were selected so that they encompassed a range from less than to greater than the K_m values estimated from preliminary experiments. The extraneuronal uptake of both isomers of each amine approached saturation (Figure 1; and plots of s/v versus s were linear, correlation coefficients $>$ 0.996). The mean K_m values obtained from these data are shown in Table 2. The K_m values of the (+)-isomers were not significantly different from each other, but the K_m of (−)-isoprenaline $<$ (−)-adrenaline $<$ (−)-noradrenaline. Thus, the order of the *ratios* of the K_m values of the (+):(−)-isomers was isoprenaline $>$ adrenaline $>$ noradrenaline (4.9, 2.5 and 1.6 respectively).

The diffusional entry was not saturable for any of the amines or isomers in that plots of v versus s (Figure 1) were linear (correlation coefficients $>$ 0.995). The slopes of the v versus s plots for the (−)- and (+)-isomers of each amine were not significantly different (Student's t test), so diffusional entry was not influenced by the isomeric form. The absolute values for diffusional entry of the three amines cannot be directly compared because of differences in the intensities of their fluorophores (Bryan & O'Donnell, 1980b), but Figure 1 shows clearly that the contribution of diffusion to the total uptake is much greater for isoprenaline than for adrenaline or noradrenaline, whether considering the (−)- or the (+)-isomer.

Discussion

In the present study, the extraneuronal uptake of both the (−)- and the (+)-isomers of noradrenaline, adrenaline and isoprenaline has been studied in guinea-pig trachealis smooth muscle cells. The initial rate of uptake of the (−)-isomer was always greater than that of the (+)-isomer, reflecting a difference between the affinities (inversely related to K_m values) of the (−)- and (+)-isomers of the amines for extraneuronal uptake. This difference was small for noradrenaline but was increased for adrenaline (N-methyl substituent) and increased again for isoprenaline (N-isopropyl substituent). Thus, there is stereoselectivity of the extraneuronal uptake of catecholamines, but it is greatest for isoprenaline and almost negligible (less than 2 fold) for noradrenaline. This agrees with some recent findings in rat heart and rabbit aorta (Grohmann *et al.*, 1983). However, in the experiments in those preparations, it was possible that the differences observed between the three amines could be ascribed to differences in their vascular effects, i.e. vasodilatation with isoprenaline and vasoconstriction with noradrenaline. Such differences were not a complicating factor in the present experiments, since guinea-pig tracheal preparations

relax to all three catecholamines. The stereoselectivity of the extraneuronal uptake of isoprenaline would provide an explanation for the observation that the rates of formation of the *O*-methylated metabolite in the rabbit aorta were greater in tissues incubated in (−)- than in (+)-isoprenaline (Barone *et al.*, 1983).

In an earlier study, we showed that the order of the affinities of catecholamines for extraneuronal uptake was adrenaline > noradrenaline = isoprenaline (Bryan & O'Donnell, 1980b). However, this conclusion was based on data for (−)-noradrenaline and (−)-adrenaline but for (±)-isoprenaline, since we assumed that extraneuronal uptake was not stereoselective. From the data obtained in the present study the order of the affinities of the (−)-isomers of the amines for extraneuronal uptake is isoprenaline > adrenaline > noradrenaline. Note that these data had also been adjusted to allow for some entry of the amines, particularly isoprenaline, by diffusion, which has recently been shown to be important in the cells being studied (Bryan *et al.*, 1984). The revised order of affinities agrees with recent results obtained in rat heart, also with the (−)-isomers (Grohmann *et al.*, 1983), and also supports early conclusions (Gillespie, 1976), even though these were based on studies with (−)-adrenaline and (−)-noradrenaline (Iversen, 1965) but with (±)-isoprenaline (Callingham & Burgen, 1966).

The present study also provided further data on the corticosterone-resistant entry of amines into guinea-pig trachealis smooth muscle cells. For both isomers of all three amines, this entry was not saturable. It was also the same for both isomers of each amine and it correlated with the lipophilicity of the amines as determined by Mack & Bönisch (1979), i.e. it was marked for isoprenaline and least for noradrenaline. Thus, we substantiated our previous conclusion that the corticosterone-resistant entry is not carrier-mediated but occurs by diffusion (Bryan *et al.*, 1984).

In conclusion, extraneuronal uptake can show stereoselectivity for the (−)-isomers of catecholamines, but there is variation between the three amines studied, in that stereoselectivity is more marked for isoprenaline than for adrenaline or noradrenaline. The K_m values obtained in the present study indicated that, when the optically active centre of the catecholamine molecule was in the (−)-configuration, a change in the nitrogen substituent from hydrogen in noradrenaline to an alkyl substituent in adrenaline and isoprenaline increased the affinity of the amine for the membrane carrier. However, the same changes in structure of the (+)-isomers of the amines did not affect their affinity for the carrier. These results suggest that the N-alkyl substituent can interact with the carrier when the optically active centre of the amine molecule is in the (−)-configuration, but not in the (+)-configuration, and also leads to the greater stereoselectivity of isoprenaline than of noradrenaline for extraneuronal uptake. Thus, in studies on extraneuronal uptake of various catecholamines, the (−)-isomer, rather than the racemate, should be selected in the future. This is possible when techniques requiring only unlabelled compounds are being used, e.g. fluorescence histochemistry or high performance liquid chromatography. However, there is at present no commercial source either of the tritium labelled (−)-isomer of isoprenaline or of the (−)-isomer of adrenaline with a tritium label in an appropriate position for extraneuronal uptake and metabolism studies. The present study has confirmed that, of the three amines studied, (−)-isoprenaline has the highest affinity for extraneuronal uptake, but since it also enters cells by diffusion to a much greater extent than either adrenaline or noradrenaline, the latter amines may be preferable to isoprenaline for extraneuronal uptake studies.

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