(Arita et al 1970; Wallace & Barnett 1978; Dyer et al 1981). The low permeation rates of dissociated compounds through skin are assumed to be due to a relative inability of ions to partition into the lipid phases of the horny layer. The lack of importance of lipid phases as a medium of transport across the nail, as suggested previously (Walters et al 1983), is reflected in the ability of both the dissociated and the undissociated species of miconazole to permeate at near equivalent rates (Fig. 1). Miconazole is a weak base with a pK_a of 6·65 and, therefore, the more acidic the medium the greater is the degree of ionization. Yet the flux of miconazole, through different nail plates, is invariant at low pH where ionization is near complete. Moreover, the permeability coefficients of the reference compound, ethanol, follow the same pattern as a function of pH. The ratio, $P_{miconazole}/P_{ethanol}$ is essentially invariant. Thus the ionic form of miconazole dissolves as easily in the nail plate as the free base. Since there is little or no dependency of permeability on pH, these data suggest that the overriding aspect in increasing topical bioavailability of miconazole, for the treatment of onychomycoses, is increasing the solubility of the drug in a formulation, which can be done by decreasing the pH.

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The affinity and efficacy of naturally occurring catecholamines at β-adrenoceptor subtypes

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The contribution of affinity and efficacy to the agonistic actions of the naturally occurring catecholamines, (−)-noradrenaline and (−)-adrenaline at β-adrenoceptor sites were assessed in guinea-pig driven left atrial (β_1) and K^+-depolarized uterine (β_2) preparations. The dissociation constants of each agonist, required in these calculations, were calculated using radioligand binding techniques. [^{125}I]Iodocyanopindolol bound to sites in membrane preparations of each tissue which have been shown to represent β_1-(atria) and β_2-(uterus) adrenoceptors. It was found that (−)-noradrenaline was approximately 10-fold more selective for the β_1- as opposed to the β_2-adrenoceptor in the pharmacological studies. Affinity/efficacy calculations indicated that this selectivity was entirely due to a selective affinity for the β_1-adrenoceptor subtype. (−)-Noradrenaline, (−)-adrenaline and the reference compound (−)-isoprenaline all had approximately the same efficacy at either β-adrenoceptor subtype.

As noted by Lands et al (1967) in their work on the subclassification of β-adrenoceptors, the naturally occurring catecholamines, (−)-noradrenaline and (−)-adrenaline display a marked difference in their potency at so called β_1- and β_2-adrenoceptors. Thus at β_1-adrenoceptors (e.g. heart) (−)-noradrenaline has a similar potency to (−)-adrenaline whereas at β_2-adrenoceptors (e.g. blood vessels) (−)-adrenaline is considerably more potent than (−)-noradrenaline as an agonist.

The purpose of this study was to ascertain whether the differing relative potencies of (−)-noradrenaline and (−)-adrenaline result from differences in affinity (i.e. different abilities of the agonists to form drug receptor complexes) or differing efficacies (i.e. different abilities of the drug receptor complex to initiate pharmacological activity). These studies were performed using the non-selective β-adrenoceptor agonist (−)-isoprenaline as the reference agonist.

Methods

Details of the methods used in the present study have been described previously (McPherson et al 1984). Guinea-pig left atrial and uterine tissues were used since previous pharmacological (Broadley 1982; Krstew et al 1982; McPherson et al 1984) and radioligand binding studies (McPherson et al 1984) have indicated the presence of homogeneous populations of β_1- and

* Correspondence.

β_2-adrenoceptors respectively. Comparisons of radioligand binding studies using the radioligand (−)-[^{125}I]iodocyanopindolol ([^{125}I]CYP) and pharmacological experiments in which positive inotropic and uterine relaxant effects were monitored have shown that the binding sites and pharmacological receptors have common characteristics (McPherson et al 1984).

Dissociation constants for the agonists (−)-noradrenaline, (−)-adrenaline and (−)-isoprenaline were determined from displacement studies in left atrial and uterine membranes using the radioligand [^{125}I]CYP. Propranolol (1 μM) was used to define specific ligand binding. Data were analysed using the computer program EBDA (McPherson 1983a, b) which performs preliminary Scatchard, Hill and Hofstee analyses and creates a file for the LIGAND program (Munson & Rodbard 1980) which was used to obtain final parameter estimates.

In organ-bath experiments, positive inotropic effects in driven left atrial preparations (2·5 Hz) and relaxant effects in K^+-depolarized uterine preparations were assessed in response to the cumulative addition of the three catecholamines. Tissues were pretreated with phenoxybenzamine to preclude interference from possible α-adrenoceptor-mediated effects and neuronal and extraneuronal uptake mechanisms. In these experiments, reproducible cumulative concentration-effect curves to (−)-isoprenaline were first obtained and thereafter curves to (−)-noradrenaline or (−)-adrenaline were established. Checks on tissue responsiveness were validated by the re-establishment of curves to (−)-isoprenaline following the testing of the other compounds.

Fractional receptor occupancy-response curves were constructed using data from the organ-bath experiments in conjunction with dissociation constants obtained from the radioligand binding studies, and relative efficacies with respect to (−)-isoprenaline calculated (Furchgott & Bursztyn 1967; McPherson et al 1984).

Results

[^{125}I]CYP bound with high affinity to a single population of binding sites in membranes prepared from either guinea-pig left atria or uterus. The mean dissociation constants (K_D) were 20 ± 30 pM (n = 4) and 21 ± 3 pM (n = 6) for atria and uterus, respectively. Corresponding maximum density of binding site (B_{max}) values were 2·5 ± 0·4 and 0·25 ± 0·04 pmol g^{-1} wet weight of tissue. In addition Hill coefficients were not significantly different from unity in both cases indicating a lack of cooperativity in binding. (−)-Isoprenaline, (−)-noradrenaline and (−)-adrenaline totally displaced [^{125}I]CYP specifically bound to atrial (β_1) or uterine (β_2) membranes. The rank orders of potency were, in atria (−)-isoprenaline > (−)-noradrenaline = (−)-adrenaline and in uterus (−)-isoprenaline > (−)-adrenaline > (−)-noradrenaline (see Table 1). (−)-Noradrenaline was approximately 10 times more potent in displacing [^{125}I]CYP from atrial as opposed to uterine membranes thus suggesting that it displays selectivity for binding sites with the characteristics of β_1-adrenoceptors. Slope factors (Hill coefficients) for the displacement curves were not significantly different from unity, indicating that each agonist, under the conditions of the binding assay, interacted with a single class of binding sites (Table 1). This was confirmed when each curve was analysed using LIGAND since only single site fits could be achieved.

In guinea-pig atrial and uterine preparations concentration-effect curves to the three catecholamines

Table 1. Organ bath and radioligand binding data for the catecholamine's actions on β_1- and β_2-adrenoceptors from the guinea-pig. In the organ bath section (1) values given include mean pD_2 (−log (EC50)), intrinsic activity (α: (−)-isoprenaline = 1) values and selectivity index (SI: EC50 (atria)/EC50 (uterus)). In the radioligand binding section (2) values are the mean pK_D (−log (K_D)), slope factor (SF = Hill coefficient) and selectivity index (SI: K_D (atria)/K_D (uterus)). Relative efficacy calculations are given in the last section (3). Values given are those relative to (−)-isoprenaline ($\varepsilon_{ISO}/\varepsilon_{drug}$: see McPherson et al 1984). All values are the mean ± s.e.m. for the specified number of experiments (n).

	Atria (β_1)			Uterus (β_2)			
1. Organ bath							
	n	pD_2	α	*n*	pD_2	α	SI
(−)-isoprenaline	4	8·31 ± 0·07	1	5	8·22 ± 0·06	1	1·2
(−)-adrenaline	4	6·82 ± 0·06	1·01 ± 0·02	4	6·97 ± 0·11	1·14 ± 0·02	0·7
(−)-noradrenaline	4	7·32 ± 0·12	0·98 ± 0·01	5	6·31 ± 0·15	0·96 ± 0·10	10·2
2. Radioligand binding							
	n	pK_D	SF	*n*	pK_D	SF	SI
(−)-isoprenaline	6	6·50 ± 0·06	1·04 ± 0·04	5	5·98 ± 0·06	0·95 ± 0·09	3
(−)-adrenaline	3	5·24 ± 0·13	1·00 ± 0·04	3	5·25 ± 0·04	1·02 ± 0·03	1
(−)-noradrenaline	3	5·34 ± 0·05	0·84 ± 0·11	3	4·37 ± 0·07	1·09 ± 0·06	9·3
3. Relative efficacy							
	n	$\varepsilon_{ISO}/\varepsilon_{drug}$		*n*	$\varepsilon_{ISO}/\varepsilon_{drug}$		
(−)-isoprenaline	—	1		—	1		
(−)-adrenaline	4	1·65 ± 0·27		4	2·86 ± 0·95		
(−)-noradrenaline	4	0·67 ± 0·11		5	2·26 ± 1·41		

were parallel and similar maximal responses were obtained. The rank orders of potency of the compounds in atrial and uterine preparations were the same as those obtained in the binding studies and (−)-noradrenaline displayed a similar selectivity for β_1- as opposed to β_2-receptor mediated actions (Table 1).

Using the dissociation constants obtained in the radioligand binding experiments together with the

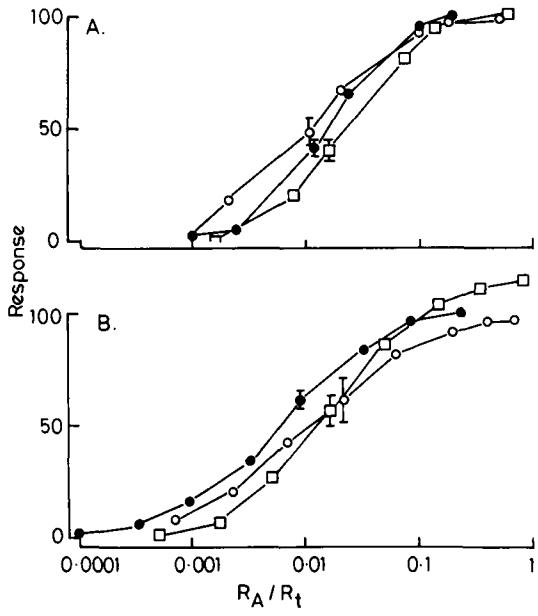

FIG. 1. Mean fractional receptor occupancy (R_A/R_t: log scale) vs response curves for (−)-isoprenaline (●), (−)-noradrenaline (○) and (−)-adrenaline (□) in the guinea-pig atria (panel A.) and guinea-pig uterus (panel B.). Responses are expressed as a percentage of the maximal response to (−)-isoprenaline in each experiment. Error bars have only been included on points near the mid-point of each curve to aid clarity. All other errors were of a similar or smaller magnitude.

results of the organ-bath studies, relative efficacies of (−)-noradrenaline and (−)-adrenaline with respect to (−)-isoprenaline were calculated. In both the atria and uterus the agonists possessed a similar efficacy (see Fig. 1) as evidenced by their close relative efficacy constants (Table 1) which are not significantly different from unity ($P < 0.05$).

Discussion

The rank orders of potency of (−)-isoprenaline, (−)-adrenaline and (−)-noradrenaline in guinea-pig atrial and uterine tissues are consistent with the actions of the compounds at β_1- and β_2-adrenoceptors, respectively. While (−)-isoprenaline and (−)-adrenaline displayed little selectivity for either β-adrenoceptor subtype, (−)-noradrenaline was approximately 10-fold more potent for β_1-adrenoceptor mediated actions.

Similar rank orders and selectivities were obtained in the radioligand binding studies, a feature which suggests a parallelism between the affinities of the drugs for the receptor sites and their pharmacological potencies. This idea was further confirmed by the finding that both (−)-noradrenaline and (−)-adrenaline have similar relative efficacies with respect to (−)-isoprenaline ($\varepsilon_{ISO}/\varepsilon_{drug}$ values were close to unity). These results imply that once the respective drug-receptor complexes have been formed, then all compounds have a similar ability to initiate a response. The selectivity that (−)-noradrenaline displays for β_1-adrenoceptor actions therefore results from its greater affinity for β_1- as opposed to β_2-adrenoceptors. It should also be noted that all agonists have a receptor reserve in the two tissues. This is apparent in Fig. 1 which shows that three agonists produce greater than 90% of their maximal response with less than 10% receptor occupancy (i.e. $R_A/R_t < 0.1$).

Ariëns & Simonis (1976) have put forward the hypothesis that in tissues receiving an adrenergic innervation the β-adrenoceptors present are of the β_1-subtype, while β_2-adrenoceptors are found in non-innervated tissued. This idea is compatible with the strong β_1- and weak β_2-adrenoceptor actions of transmitter noradrenaline and the potent β_2-receptor actions of circulating adrenaline, the catecholamine responsible for activity in non-innervated tissues. If the above hypothesis is generally applicable, the results of the present study might suggest that the greater sensitivity of the β_1-adrenoceptor to transmitter (−)-noradrenaline results from an adaptation of the actual receptor rather than the receptor/response coupling system.

In conclusion the results of this study indicate that the greater activity of (−)-noradrenaline at β_1-adrenoceptor sites in the preparations used, results from a greater affinity of (−)-noradrenaline for this β-adrenoceptor subtype. (−)-Noradrenaline, (−)-adrenaline and (−)-isoprenaline have similar efficacies at both β_1- or β_2-adrenoceptors.

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