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Role of efficacy in the assessment of the actions of α-adrenoceptor agonists in rat aorta with endothelium

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Concentration-effect curves to phenylephrine are shifted to the right in the presence of endothelium in rat aorta while responses to clonidine are practically abolished. Analysis of the concentration-effect curves showed that the effect of endothelium could possibly be explained by a reduction in relative intrinsic efficacy of the two agonists by between 3·5- and 6-fold. Published observations on the modulatory effects of agonist-induced contractions by endothelium in the rat aorta tend to support this explanation. It is further concluded that this reduction in efficacy could be related to the basal release of an endothelium-derived substance and that changes in tissue contractility in the presence of endothelium cannot necessarily be taken as evidence for a stimulated liberation of endothelium-derived products by agonists.

Since Furchgott & Zawadzki (1980) first described the endothelial-dependent relaxant effect of acetylcholine in rabbit aorta, it has been established that the vascular endothelium can mediate, wholly or partially, the relaxant responses to a large number of agonists (Furchgott 1984). This effect of endothelium is thought to be due to the stimulated liberation of an as yet unidentified substance or substances, with a short half life of about 6 s (Griffith et al 1984). It has also been shown that endothelium markedly modifies agonist-induced contractile responses (Allan et al 1983; Cocks & Angus 1983: Eglème et al 1984; Lues & Schümann 1984). The presence of endothelium reduces maximal contractile responses and increases the EC50 values for concentration-effect curves to clonidine, oxymetazoline, UK-14,304 (2-(8-bromoquinoxalyl-7-imino)imidazolidine) and B-HT 920 (2-amino-6-allyl-5,6,7,8-tetrahydro-4*H*-thiazolo[5,4-*d*]azepine dihydrochloride) in rat isolated aortic preparations, whilst curves to phenylephrine, noradrenaline, guanfacine, methoxamine and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) are displaced to the right with less marked changes in their maximal effects (Eglème et al 1984; Bigaud et al 1984; Godfraind & Miller 1984; Miller et al 1984; Lues & Schümann 1984; Godfraind et al 1985). On the basis of these observations is the hypothesis that in general the α_2-adrenoceptor-selective agonists are more sensitive to the effects of an intact endothelium and that they, and other adrenoceptor agonists, may provoke the release of an endothelial factor or factors by an interaction with α_2-adrenoceptors located on the endothelial cell (Cocks & Angus 1983; Miller et al 1984). However, in rat aorta the α_2-adrenoceptor-selective agonist, guanfacine does not behave identically to clonidine (Godfraind et al 1985) and the α_1-adrenoceptor agonist St 587 (2-(2-chloro-5-trifluoromethylphenylimino)imidazoline) does not behave identically to noradrenaline (Lues & Schümann 1984).

An alternative explanation to interaction with a specific receptor type on the endothelial cells can be proposed, based on the concepts of efficacy and receptor reserves, if it is assumed that the presence of endothelial cells does not alter the affinity of agonists for their respective receptor sites. For this explanation to be plausible it is essential that the α-adrenoceptor agonists typified by phenylephrine have a higher efficacy (i.e. larger receptor reserve) than agonists such as clonidine, which should have low efficacy and preferably no receptor reserve. Under these conditions it can be shown theoretically that the presence of endothelial cells reduces the efficacy of agonists by a similar degree and that this results in the observed concentration-effect curves for compounds in the two groups typified by phenylephrine and clonidine. Thus, the aim of the present study was to examine this possibility, by applying experimental data obtained from organ bath experiments with

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phenylephrine and clonidine to the accepted models of drug-receptor interaction and to compare finally the observed and predicted curves for the two drugs in the presence of endothelium.

METHODS AND RESULTS

Paired rings of thoracic aorta of female Wistar rats (12 to 15 weeks old), with or without endothelium, were suspended under 2 g tension in Krebs solution maintained at 37 °C and aerated with a mixture of 95% O_2 and 5% CO_2. Curves were established using cumulative additions of drugs and contractions were recorded isometrically (Bigaud et al 1984; Miller et al 1984). In the presence of endothelium, acetylcholine (10^{-6} M) relaxed phenylephrine-induced maximal contractions by 75 ± 3% and abolished clonidine contractions. In the absence of endothelium, acetylcholine had no significant effect on maximal contractions elicited by either agonist.

Concentration-effect curves to phenylephrine and clonidine in the presence and absence of endothelium are shown in Fig. 1a. The mean (±s.e.m.) EC50 values for phenylephrine and clonidine in the absence of endothelium were $4{\cdot}9 \pm 0{\cdot}4 \times 10^{-8}$ (n = 5) and $2{\cdot}0 \pm 0{\cdot}6 \times 10^{-8}$ M (n = 4), whilst in the presence of endothelium the values were $1{\cdot}4 \pm 0{\cdot}1 \times 10^{-7}$ (n = 5) and $2{\cdot}2 \pm 0{\cdot}9 \times 10^{-7}$ M (n = 4), respectively. Clonidine elicited about the same maximal response as phenylephrine in the absence of endothelium but its response was reduced to about 17% of maximum in the presence of endothelium.

The dissociation constant of the agonist (K_a) is used in the computation of efficacy so it is essential to use the most appropriate determination available. As the nature of the α-receptor in the rat aorta may differ from other α-receptors (Ruffolo & Waddell 1982; Randriantsoa et al 1981), the K_a values for phenylephrine ($3{\cdot}5 \times 10^{-7}$ M) and clonidine ($2{\cdot}0 \times 10^{-8}$ M) were taken from organ bath studies conducted on rat isolated aortic preparations (see Ruffolo et al 1979 for methods). Briefly, the K_a value for phenylephrine obtained by Ruffolo et al (1979) was determined from analysis of a pair of concentration-effect curves established in the absence of, and after, irreversible inactivation of a proportion of the α-receptor population by dibenamine. The K_a value for clonidine, as determined by Ruffolo et al (1979), was calculated from clonidine-induced rightward shifts of superimposed phenylephrine curves following reduction of the intrinsic activity of the imidazole by treatment of tissues with low concentrations of dibenamine. The receptor occupancy curves for the two agonists, calculated from the law of mass action using these K_a values (Furchgott & Bursztyn 1967), are also plotted in Fig. 1.

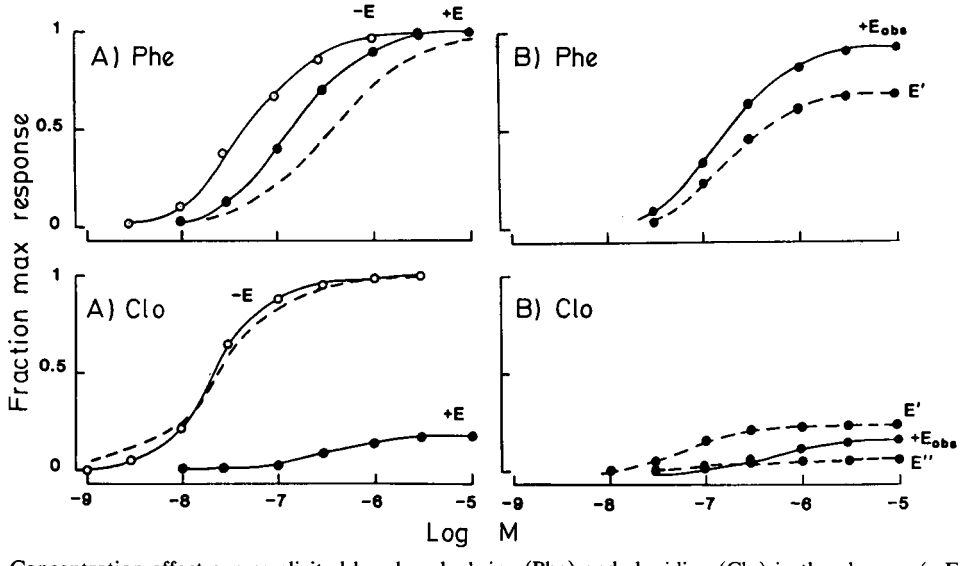

FIG. 1. A. Concentration-effect curves elicited by phenylephrine (Phe) and clonidine (Clo) in the absence (−E, open symbols) and presence (+E, filled symbols) of endothelium (solid lines). Dotted lines represent the calculated proportion of receptors occupied at each concentration of agonist (see text for details). B. Experimental concentration-effect curves elicited by phenylephrine and clonidine in the presence of endothelium (solid lines) compared with expected concentration-effect curves calculated by assuming that the presence of the endothelium reduces the efficacy of the agonists by 3·5-fold (E′) or by 6-fold (E″), respectively. See text for further explanation.

Inspection of the receptor occupancy and response curves for each agonist indicates that while the 'receptor reserve' for phenylephrine is reduced by the endothelium, it is not abolished. It is evident that clonidine possesses no receptor reserve in the absence of endothelium.

The relative intrinsic efficacies (E) of the drugs were calculated as described by Furchgott & Bursztyn (1967) using equieffective points corresponding to 5, 10 and 12·5% of the maximal responses to the drugs in the absence of endothelium. In this procedure log (concentration)-effect curves are converted to log (fraction receptor occupancy)-effect curves using the equation:

$$\text{Fraction of receptors occupied} = A/(A + K_a)$$

where A is the concentration of agonist and K_a its dissociation constant. For equal responses it can be shown that the antilog of the distance between points on the curves for a reference agonist, phenylephrine, and another agonist (clonidine) is a measure of the relative intrinsic efficacy between the two drugs (see Furchgott & Bursztyn 1967, for full description).

In the absence of endothelium, the intrinsic efficacy of phenylephrine was 8·5 times that of clonidine, whilst in the presence of endothelium the E value for phenylephrine was 17·5 times that of clonidine. The low levels of response to clonidine in the presence of endothelium, necessitating the use of 5, 10 and 12·5% response levels, precludes a more accurate assessment of the relative intrinsic efficacy. However, the results show that the endothelium appears to reduce the efficacy of the two agonists to a similar degree. This conclusion is supported by calculation of the relative intrinsic efficacy of each agonist from their respective concentration-effect curves in the absence and presence of endothelium which show that the presence of endothelium reduces the intrinsic efficacy of phenylephrine by about 3·5-fold and of clonidine by about 6-fold.

The inverse of the fraction of receptors occupied in producing a half-maximal response to the agonist offers an alternative measure of the relative changes in the efficacy of an agonist. In this system, for example, a drug which occupies 0·02 of the total receptor population at the EC50 level is assigned an 'efficacy' of 50. Using the EC50 values and K_a values quoted above, phenylephrine and clonidine have efficacies of 8·08 and 2·00, respectively, in the absence of endothelium, while in the presence of endothelium the corresponding values are 3·48 and 1·09, respectively. Thus for phenylephrine there is a 2·32-fold and for clonidine a 1·83-fold change in efficacy in the presence of endothelium. These results therefore support the above conclusion that the endothelium appears to reduce the efficacy of the two agonists to a similar degree.

The absolute efficacies for phenylephrine and clonidine were calculated from their dose-response curves in the absence of endothelium as the final aspect of this study. Theoretical concentration-response curves for the agonists were then calculated following a reduction in their respective efficacies and the resulting curves compared with the experimental curves observed in preparations with endothelium.

Although the absolute real value of efficacy cannot be determined, a value for the absolute efficacy (e) can be calculated using the equation:

$$S = e.(A)/[(A) + K_a]$$

by assuming that the stimulus (S) equals unity when the response is half maximal, i.e. when the concentration (A) is equal to the EC50 value and K_a is the dissociation constant.

With this procedure, the values of e were 8·4 and 2 for phenylephrine and clonidine, respectively.

With the same values of e, values of S were calculated for each concentration of agonist and these were plotted as a function of the observed response (i.e. response vs stimulus plot).

For the purposes of the present calculations, the efficacies of phenylephrine and clonidine were decreased 3·5-fold and 6-fold, respectively, as calculated by the method of Furchgott & Bursztyn (1967) (see above).

Values of S were subsequently recalculated using values of e reduced 3·5- and 6-fold to simulate the presence of endothelium. Assuming no alteration in the stimulus-response relationship, these new values of S were used with the response vs stimulus plot to read off the expected response. In turn, the predicted value of the response R was plotted against the concentration of the agonist, to construct theoretical concentration-response curves for the agonists as if in the presence of endothelium. Fig. 1B shows both the theoretical (E′) and observed (+Eobs) concentration-response curves for the two agonists. For clonidine, theoretical curves corresponding to reductions in efficacy of both 3·5- and 6-fold are shown. The observed curve for clonidine appears to reflect a reduction in efficacy of between 3·5- and 6-fold.

A difference between the theoretical and observed curves for phenylephrine is the small depression in the maximal response of the theoretical curve. In this type of analysis the magnitude of the maximal response is highly dependent on the K_a value of the agonist. For example in this present case a 3-fold increase in the K_a value (a reduction from $3{\cdot}5 \times 10^{-7}$ to $1{\cdot}2 \times 10^{-6}$ M) would allow the theoretical curve to reach the same maximal response as in the observed situation. Nevertheless, in general, there is good agreement between the two curves, suggesting that a similar magnitude of reduction in efficacy can reproduce the curves observed for the two agonists in the presence of endothelium.

From this analysis it may be concluded that an equal reduction in efficacy for both agonists can account for the markedly different concentration-effect curves for phenylephrine and clonidine observed in the presence of endothelium. This raises the question of whether such a reduction in efficacy can explain the observed changes in the dose-response curves for other agonists in the presence of endothelium.

The extent of receptor reserves for the various agonists is all that need be considered in light of the present analysis. In this regard, comparisons of the K_a values and EC50 values for agonists in rat aorta preparations in the absence of endothelium would provide an index for predicting such reserves of receptors. For noradrenaline and guanfacine, their respective EC50 values ($6{\cdot}7 \times 10^{-9}$ and $2{\cdot}1 \times 10^{-7}$ M, Godfraind et al 1985; $2{\cdot}3 \times 10^{-9}$ and $8{\cdot}7 \times 10^{-8}$ M, Lues & Schümann 1984) are much lower than their respective K_a values of $2{\cdot}6 \times 10^{-7}$ (Ruffolo & Waddell 1982) and $1{\cdot}02 \times 10^{-5}$ M (Digges & Summers 1983). Therefore, these observations would predict a rightward shift of the curves without marked changes in their maximal responses in the presence of endothelium, similar to the observed curves (Eglème et al 1985; Lues & Schümann 1984; Godfraind et al 1985). However, for oxymetazoline and B-HT 920, their respective EC50 values of $4{\cdot}7 \times 10^{-7}$ (Godfraind et al 1985) and $1{\cdot}3 \times 10^{-6}$ M (Miller et al 1984; Lues & Schümann 1984) are similar to their respective K_a values of $6{\cdot}8 \times 10^{-7}$ (Ruffolo & Waddell 1982) and $4{\cdot}2 \times 10^{-6}$ M (Schini unpublished observations). Since these two agonists do not have effective receptor reserves, they would be expected to behave like clonidine when curves are established in the presence of endothelium—again similar to observed results (Miller et al 1985; Lues & Schümann 1984; Godraind et al 1985). It is therefore apparent that, in general, a reduction in efficacy can explain the two patterns of concentration-effect curves observed for a number of α-adrenoceptor agonists in the presence of endothelium.

Considerations of efficacy pose an important question. Do the α-receptor agonists provoke the release of a substance or substances from the endothelium or do the concentration-effect curves reflect the presence of a resting release of substance(s)? If tissue cyclic (c)GMP levels are taken as an indication of the release of a factor from the endothelium, then α-adrenoceptor agonists provoke a release since levels of cGMP are elevated by 1·5- to 2-fold in the presence of endothelium but are not altered in its absence (Bigaud et al 1984: Miller et al 1984). Resting levels of cGMP from aortae with endothelium are elevated 2- to 3-fold above levels measured in aortae without endothelium (see Rapoport & Murad 1983 for review; Bigaud et al 1984; Miller et al 1984). Thus, there is evidence for a basal release of endothelial factor(s) and for stimulated release by α-adrenoceptor agonists. The influence of the resting release of endothelial substance can be gauged by assessing the alterations in concentration-effect curves for $PGF_{2\alpha}$ and the calcium-entry agonist Bay K 8644 methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)pyridine-5-carboxylate) since neither of these agonists stimulate an increase in the tissue level of cGMP (Schoeffter unpublished observations; Miller et al 1985). For these two agonists, concentration-effect curves, established in the presence of endothelium, are displaced to the right of those in the absence of endothelium by 2- to 3-fold, and it is this shift which reflects the influence of resting levels of endothelial factor.

CONCLUSIONS

The conclusions that can be drawn are that if tissue levels of cGMP are related to the presence of a factor derived from the endothelium, then the α-adrenoceptor agonists do provoke release of a substance, but probably not in sufficient amounts to alter further the concentration-effect curves to agonists above that observed due to resting release of a substance or substances from the endothelium. In the presence of endothelium phenylephrine, 3×10^{-9}–10^{-6}, noradrenaline, 3×10^{-9}–10^{-6}, clonidine, 10^{-9}–3×10^{-6} and B-HT 920, 3×10^{-7}–10^{-4} M, do not elicit relaxation of rat aorta contracted by $PGF_{2\alpha}$ (3×10^{-6} M) either in the absence or presence of prazosin (10^{-7} M) (Schini unpublished observations).

If a change in efficacy is an important feature of endothelial modulation of agonist-induced responses, then it follows that a variation in the proportion of spare receptors for a particular agonist in various vessels will affect the degree of modulation by the endothelium. That is a 'tissue selectivity' of endothelium as a modulator of contractile effects to a particular agonist would be expected.

In summary, apart from the finding that a change in efficacy (in the assumed absence of a change in the affinity of agonists) can explain the effects of the endothelium on responses to α-receptors, the present analysis also brings to light another point for consideration. It is that while an agonist curve may be affected by the presence of endothelium, it is necessary to provide additional evidence to support the contention that the agonist is stimulating the release of a substance or substances from the endothelium, and that it is this enhanced release which accounts for the alteration in the dose-response curve.

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