Biochemical Pharmacology, Vol. 28, pp. 1011–1013.
Pergamon Press Ltd. 1979. Printed in Great Britain.

FACTORS CONTROLLING THE SELECTIVITY OF β BLOCKING DRUGS

A. J. COLEMAN, D. S. PATERSON and A. R. SOMERVILLE

Imperial Chemical Industries Limited, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, England

(*Received* 10 *July* 1978; *accepted* 5 *October* 1978)

Abstract—The effects of a number of isomeric pairs of β blockers on adenylate cyclase activation and [^{3}H]-dihydroalprenolol binding have been examined using membrane preparations from rabbit heart and superovulated rat corpus luteum. *Para* isomers always show decided selectivity for heart membranes whereas *meta* isomers do not. There are also differences in the relationships between K_I (cyclase) and K_D (binding) which current theory is not adequate to explain.

Many drugs are now known which can selectively affect adrenergic β receptors in certain tissues more than others, and the classification of tissues into β_1 and β_2 types by Lands, Arnold, McAuliff, Luduena and Brown [1] has been amply confirmed, in an operational sense at least. This does not necessarily imply the existence of a different receptor in each tissue however, since factors such as distribution and metabolism could result in varying concentrations of drug around identical receptors, depending on the lipid character and enzymic content of the tissue. In a previous paper [2] we have shown that membrane fragments prepared from heart and uterus, in which distribution and metabolic effects should be minimized, retain a catecholamine stimulatable adenylate cyclase which is differentially inhibited by selective β blockers. This satisfies one of the primary criteria for differentiating receptors [3] although Buckner and Abel have argued that different degrees of blockade by the same antagonist in two tissues is not a sufficient criterion of different receptors [4, 5]. They have pointed out that drugs must penetrate different barriers in different tissues to gain access to the receptors, and this implies that optical isomers of an agonist or antagonist should have the same potency ratio on two tissues if their receptors are identical. Using the optical isomers of several selective agonists, they conclude that guinea pig atria and trachea have identical receptors. They explain this unexpected finding in terms of differential tissue receptor reserve (or efficiency of receptor–cyclase coupling) [6], but their hypothesis has been disproved by O'Donnell and Wanstall [7].

Although we believe that use of membranes has removed any gross drug penetration problems, receptors may sit in a micro-environment where physical properties could control distribution of the drug between bathing fluid or the surrounding membrane and the receptor site. To eliminate such variables we have extended our work to include a number of isomeric pairs of *meta/para* substituted compounds with similar partition coefficients. As a β_2 system we have used corpus luteum membranes which we have previously shown to have β_2 characteristics in terms of cyclase stimulation by a series of agonists and blockade by selective antagonists, though with some anomalies [8]. These membranes show a 10- to 20-fold stimulation by isoprenaline as compared to 50–100 per cent for uterus, and in consequence the reproducibility of assays is much better. The *para* isomers always show decided selectivity for heart membranes whereas the *meta* isomers do not. This is true both of the inhibition of cyclase activation and the displacement of dihydroalprenolol from binding sites and we consider it to be very strong evidence for the existence of β receptor subtypes differing in molecular structure rather than purely distribution characteristics.

METHODS

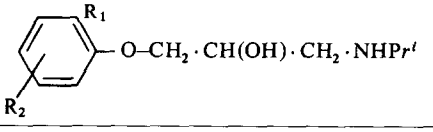
Membranes were prepared from hearts of reserpinized rabbits and corpora lutea from superovulated rats as described previously [2, 8]. Antagonism to isoprenaline stimulation of adenylate cyclase and displacement of [^{3}H]dihydroalprenolol were also measured as before [2, 8] using Schild plots to estimate K_I values for cyclase inhibition. ED_{50} values for dihydroalprenolol displacement were obtained from plots of per cent displaced against log dose, using log-probit paper, and these were converted to K_D values using the formula:

$$K_D \text{ (drug)} = \frac{ED_{50} \text{ (drug)}}{ED_{50} \text{ (alprenolol)}} \times K_D \text{ alprenolol.}$$

where the ED_{50} for alprenolol is the value measured in the same experiment and the K_D alprenolol is a mean value obtained from a number of experiments ($6.1 \pm 0.55 \times 10^{-9}$ M ($n = 20$) for heart and $3.70 \pm 0.51 \times 10^{-9}$ M ($n = 14$) for corpus luteum). K_I is used to distinguish the dissociation constant as measured by inhibition of adenylate cyclase activation from the K_D measured by displacement of dihydroalprenolol.

Partition coefficients were measured by Mr P. J. Taylor and Miss B. Ashton using standard shake flask methods.

Table 1. Dissociation constants (K_I) for *meta/para* isomers of β blockers measured on activation of membrane adenylate cyclase by isoprenaline



ICI No.	R_1	R_2	Log P*	Heart K_I (M)	Corpus luteum K_I (M)	Cardioselectivity ratio
50172 (practolol)	H	*p*-NHCOMe	0.79	3.2×10^{-6}	6.2×10^{-5}	19.4
53636	H	*m*-NHCOMe	0.74	4.7×10^{-6}	3.2×10^{-6}	0.7
58846	Me	*p*-NHCOMe	—	8.3×10^{-7}	1.4×10^{-5}	17.0
69315	Me	*m*-NHCOMe	—	5.6×10^{-6}	1.5×10^{-6}	0.3
66082 (atenolol)	H	*p*-CH_2CONH_2	0.23	1.3×10^{-6}	1.3×10^{-5}	10.0
67064	H	*m*-CH_2CONH_2	0.22	3.5×10^{-6}	1.3×10^{-6}	0.4
72860	H	*p*-$CH_2NHCOMe$	0.32	1.2×10^{-6}	5.7×10^{-6}	4.6
61265	H	*m*-$CH_2NHCOMe$	0.34	3.1×10^{-6}	1.2×10^{-6}	0.4
70267	H	*p*-$OCH_2CONHMe$	0.56	1.1×10^{-5}	2.0×10^{-5}	1.8
72530	H	*m*-$OCH_2CONHMe$	0.35	3.3×10^{-6}	1.3×10^{-6}	0.4
77163	H	*p*-NHCONHMe	—	1.5×10^{-5}	1.2×10^{-4}	7.8
77385	H	*m*-NHCONHMe	—	1.4×10^{-5}	4.2×10^{-6}	0.3

* Octanol–water.

RESULTS AND DISCUSSION

Table 1 shows the apparent dissociation constants (K_I) and cardio-selectivity ratios for a number of isomeric, *meta/para* substituted pairs of phenoxypropanolamines measured on cyclase activation by isoprenaline. Log P values (octanol–water) are shown where measured: both direct measurement and calculation give very similar values for the two members of each pair. Table 2 shows K_D values and cardioselectivity ratios for the same compounds measured by dihydroalprenolol displacement. In all cases the *para* isomers are much more cardioselective than the *meta*, whether cyclase activation or receptor binding is considered. This difference must reflect different stereochemistry of the receptors rather than distribution of the drug since the latter would be expected to be similar for isomers of similar log P. When the binding constants are plotted against those for inhibition of cyclase activation (Fig. 1), it can be seen that the relationship is not the same in both tissues since the corpus luteum values fall on a different line from those for the heart. Classical theory requires that K_D and K_I should be identical for antagonists since they are assumed to act only by strict competition at the agonist binding site. In the case of an agonist the two values may differ due to the efficacy of coupling receptor occupation to effect, and the K_D/K_I ratio has been used as a measure of efficacy [9], but provided the efficacy is a linear function and is not affected by the drug, it should not influence the K_I for an antagonist. On the log–log plot of Fig. 1 the corpus luteum line has a slope close to 1, signifying proportionality, if not identity of K_D and K_I, but the heart line

Table 2. Dissociation constants (K_D) for *meta/para* isomers of β blockers measured by direct binding (dihydroalprenolol displacement)*

ICI No.	Heart K_D (M)	Corpus luteum K_D (M)	Cardioselectivity ratio
50172 (practolol)	5.4×10^{-7}	7.2×10^{-5}	133.0
53636	8.2×10^{-7}	2.4×10^{-6}	2.9
58846	2.0×10^{-7}	1.15×10^{-5}	58.0
69315	9.7×10^{-7}	2.1×10^{-6}	2.2
66082 (atenolol)	3.7×10^{-7}	1.5×10^{-5}	40.0
67064	5.9×10^{-7}	3.2×10^{-7}	0.5
72860	2.8×10^{-7}	4.6×10^{-6}	17.0
61265	8.0×10^{-7}	1.2×10^{-6}	1.5
70267	9.1×10^{-7}	4.8×10^{-5}	38.0
72530	5.9×10^{-7}	1.6×10^{-6}	2.8
77163	2.3×10^{-6}	5.8×10^{-5}	25.0
77385	2.7×10^{-6}	6.1×10^{-6}	2.0

* For structures see Table 1.

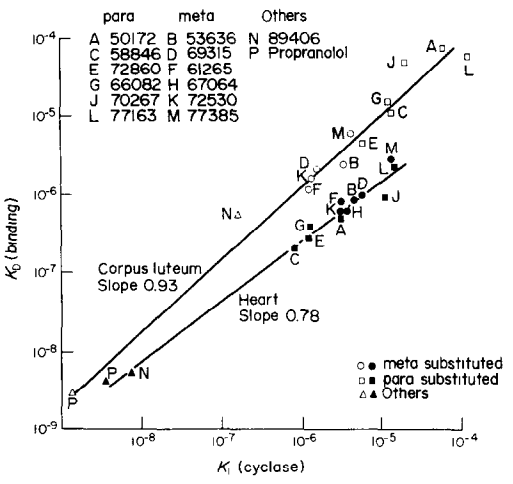


Fig. 1. Correlation of binding (K_D—displacement of [^{3}H]-DHA) and cyclase (K_I—inhibition of isoprenaline activation) constants for selective and non-selective β blocking drugs. Slopes calculated by a non-linear least squares method.

has a lower slope which is significantly different from 1 ($P < 0.001$, slopes estimated by a non-linear least squares method). While bearing in mind the warnings of Maguire *et al.* [9] about the need to compare K_D and K_I under identical conditions, we feel that any variations between binding and cyclase measurements should be constant for the two tissues and hence that this difference in slopes is real, but at present we have no explanation for it.

It can also be seen from these graphs that the *meta* isomers are generally more potent than the *para* in corpus luteum, while in heart the potencies of the two isomers are more randomly distributed, suggesting that the heart receptor can accommodate both isomers equally well and that the selectivity of the *para* isomers is accounted for mainly by the discrimination of the β_2 receptor.

In summary, the evidence presented here indicates the existence of a stereochemical difference between the β_1 and β_2 receptors and suggests that there may also be differences between the tissues with respect to the coupling of receptors to cyclase in a way that is not yet understood.

Acknowledgements—We are indebted to Dr R. A. Ferguson for statistical calculations, and it is a pleasure to acknowledge the technical assistance of Mr C. D. Adams.

REFERENCES

1. A. M. Lands, A. Arnold, J. P. McAuliff, F. P. Luduena and T. G. Brown Jr, *Nature, Lond.* **214**, 597 (1967).
2. A. J. Coleman and A. R. Somerville, *Br. J. Pharmac.* **59**, 83 (1977).
3. R. F. Furchgott, *Ann. N. Y. Acad. Sci.* **139**, 553 (1967).
4. C. K. Buckner and P. N. Patil, *J. Pharmac. exp. Ther.* **176**, 634 (1971).
5. C. K. Buckner and P. Abel, *J. Pharmac. exp. Ther.* **189**, 616 (1974).
6. C. K. Buckner and R. K. Saini, *J. Pharmac. exp. Ther.* **194**, 565 (1975).
7. S. O'Donnell and J. Wanstall, *Br. J. Pharmac.* **60**, 255 (1977).
8. A. J. Coleman, D. S. Paterson and A. R. Somerville, *Biochem. Pharmac.* **28**, 1003 (1979).
9. M. E. Maguire, E. M. Ross and A. G. Gilman, *Advances in Cyclic Nucleotide Research*, (Eds P. Greengard and G. A. Robison) Vol. 8, p. 6. Raven Press, New York (1977).