

BIOCHEMICAL EVIDENCE FOR THE DUAL ACTION OF LABETALOL ON α - AND β -ADRENOCEPTORS

MARTINE AGGERBECK, GEORGES GUELLAEN & JACQUES HANOUNE

Unité de Recherches U-99, Hôpital Henri Mondor, 94010 Créteil, France

- 1 Labetalol (AH 5158A) inhibited the adrenaline-stimulated adenylate cyclase activity of rat liver and heart. This drug had no effect on basal or guanosine triphosphate (GTP)-activated adenylate cyclase activities.
- 2 Labetalol displaced the binding of the specific ligands [^3H]-dihydroergocryptine and (-)-[^3H]-dihydroalprenolol from their respective α and β -adrenoceptors in rat heart and liver. The affinity of labetalol was 10 fold higher for the β - than for the α -adrenoceptor. It appeared to be 10 to 100 times less potent than phenolamine in blocking α -adrenoceptors and 5 to 10 times less potent than propranolol in blocking β -receptors.
- 3 It is concluded that labetalol exerts its dual α - and β -antagonism by acting directly on the plasma membranes, where it binds competitively to α - and β -adrenoceptors.

Introduction

Labetalol (AH 5158A) has been reported to be the first, mixed α - and β -antagonist according to pharmacological criteria (Farmer, Kennedy, Levy & Marshall, 1972; Brittain & Levy, 1976; Richards, 1976) and, consequently, is now investigated as a therapeutic agent for arterial hypertension (Koch, 1976; Dargie, Dollery & Daniel, 1976). However, up to now, there has been no biochemical approach to the dual action of this drug. Thus, the aim of the present work was to provide experimental evidence for the direct action of labetalol on the α - and β -adrenoceptors.

The interactions of agonist catecholamines with β -adrenoceptors are known to activate adenylate cyclase (EC 4.6.1.1.) (Sutherland, Oye & Butcher, 1965). We have, therefore, studied the effect of labetalol upon the production of cyclic adenosine 3',5'-monophosphate (cyclic AMP) *in vitro* as a test for its interaction with the β -adrenoceptor. In contrast, no simple biochemical event can be studied *in vitro* as a measure of the α -adrenoceptor occupancy. However, it is now possible to assess the direct interaction of any drug with the α - and β -adrenoceptor sites by its competitive effect on the binding of [^3H]-dihydroergocryptine ([^3H]-DHEC) and (-)-[^3H]-dihydroalprenolol ((-)-[^3H]-DHA) respectively (Alexander, Williams & Lefkowitz, 1975; Williams, Mullikin & Lefkowitz, 1976). Our results demonstrate that labetalol acts directly at the plasma membrane level in liver as well as in heart, by binding to both the α - and β -adrenoceptors.

Methods

Preparation of purified liver plasma membranes

Plasma membranes were prepared from the liver of adrenalectomized, female Wistar rats (about 100 g body weight) according to the procedure devised by Neville (1968) up to step 11. (We have shown previously (Leray, Chambaut, Perrenoud & Hanoune, 1973) that adrenalectomy enhanced the sensitivity of liver adenylate cyclase to catecholamines *in vitro*. Once adrenalectomized, rats received 0.15 M NaCl instead of drinking water 6 to 8 days before they were killed.) The purified membrane preparations were suspended in 1 mM NaHCO₃ and stored in liquid nitrogen until use. The same membrane preparation was used for adenylate cyclase assay and binding studies.

Preparation of particulate fraction from heart

Particulate fractions from heart used for adenylate cyclase assay were prepared by homogenization of a 1 g fraction of tissue in 11 ml of 1 mM NaHCO₃ with a loose-fitting Dounce homogenizer. The fractions were washed twice by suspension in 1 mM NaHCO₃ and centrifugation at 2000 *g* for 10 min at 4°C. The pellets enriched with plasma membranes were resuspended in 3 volumes of 1 mM NaHCO₃ and stored in liquid nitrogen until use. A more purified membrane preparation, obtained according to Alexander *et al.* (1975) was used for the binding assays

of (-)-[³H]-DHA and [³H]-DHEC to the heart receptor sites.

Binding assays

The binding assay of [³H]-DHEC was carried out as described by Williams *et al.* (1976) with slight modifications. Unless otherwise stated, 5 nM [³H]-DHEC (12,000 ct min⁻¹ 100 µl⁻¹) and rat liver plasma membranes (1 mg membrane protein/ml) or rat heart membranes (0.5 mg membrane protein/ml) were incubated with constant shaking for 10 min at 30°C in 50 mM Tris-HCl pH 7.2 containing 10 mM MgCl₂. The final volumes of incubation were 0.4 and 0.25 ml in presence of liver membranes or heart membranes respectively. The drug was dissolved in ice cold water and added to the assay just before the experiment. At the end of the incubation, triplicate 100 µl or 75 µl aliquots (according to the final volumes of incubation medium) were diluted with 4 ml of ice cold buffer and immediately filtered through Whatman GF/C glass fibre filters (24 mm diameter). The filters were immediately washed with 15 ml of incubation buffer at 4°C, dried, placed in scintillation vials, eluted with 1 ml of methanol, and counted in 10 ml ACS aqueous scintillation mixture (Amersham Searle) at an efficiency of 40%.

In the present experiments, specific binding refers to the fraction of bound [³H]-DHEC displaced by 0.1 mM phentolamine which represented 75 to 80% of the radioactivity retained on the filter. In all the results expressed as '[³H]-DHEC bound', only the specific binding was considered.

Binding of (-)-[³H]-DHA (5 nM) to rat liver plasma membranes (about 1.2 mg protein/ml) or rat heart membranes (about 0.7 mg protein/ml), was carried out under the same conditions. Specific binding was defined as the fraction which was displaced by 0.05 mM (±)-alprenolol in the heart, or by 0.05 mM (±)-alprenolol plus 0.1 mM phentolamine in liver. As previously reported in other systems (Sporn & Molinoff, 1976; Brown, Fedak, Woodard, Aurbach & Rodbard, 1976; Schmitt & Pochet, 1977) the presence of the α-adrenoceptor antagonist, phentolamine, decreased the non-specific binding by 40%.

Adenylate cyclase assay

Adenylate cyclase activity was measured as previously reported (Hanoune, Lacombe & Pecker, 1975; Hanoune, Stengel, Lacombe, Feldmann & Coudrier, 1977). Incubation was initiated by addition of the membranes and was performed for 10 min in a shaking water bath at 33°C. Reactions were terminated by a modification of the procedure used by White (1974): samples were mixed with 0.2 ml of 0.05 N HCl, boiled for 6 min, buffered with 0.2 ml of 1.5 M imida-

zole and finally applied to alumina columns. Cyclic AMP was then eluted with 3 ml of 10 mM imidazole pH 7.5. Results are expressed as nmol of cyclic AMP formed in 10 min per mg protein. The results obtained from triplicate determinations, agreed within ±5%. In all experiments, proteins were measured according to Lowry's procedure using bovine serum albumin as standard.

Drugs

Labetalol (AH 5158A, Glaxo Evans), butoxamine (Burroughs Wellcome), phentolamine, (±)-alprenolol (Ciba-Geigy), practolol, (-)-propranolol (ICI) were obtained as gifts. (-)-Adrenaline, (-)-isoprenaline (Sigma), GTP (Serva), creatine kinase (Boehringer Mannheim), creatine phosphate (Calbiochem) were from the commercial sources indicated. All other chemicals were of analytical grade and purchased from Merck (Darmstadt).

Cyclic [8-³H]-AMP (13 Ci/mmol) was obtained from the C.E.A. (Saclay, France). [α-³²P]-ATP (21.5 Ci/mmol), [³H]-dihydroergocryptine (24 Ci/mmol) and (-)-[³H]-dihydroalprenolol (33 Ci/mmol) were from New-England Nuclear Corp. The purity of the last two compounds was routinely checked by thin layer chromatography on silica gel plates. The solvent systems were: chloroform:benzene:ethanol (4:2:1, v/v/v) for [³H]-DHEC (*R_F*: 0.46) and *n*-butanol:acetic acid:water (12:2:5, v/v/v) for [³H]-DHA (*R_F*: 0.53). Purity was greater than 95% in both cases.

Results

Effects of labetalol on rat liver adenylate cyclase activity

Since activation of the catalytic unit of adenylate cyclase systems can serve as a measure of β-adrenoceptor function, we first assessed the action of labetalol on this enzyme in liver plasma membrane. As depicted in Figure 1, basal adenylate cyclase activity (0.65 nmol cyclic AMP formed in 10 min per mg protein) as well as that stimulated by 10 µM guanosine triphosphate (GTP) (0.95 nmol cyclic AMP formed in 10 min per mg protein) were unaffected by concentrations of labetalol from 10 nM to 0.1 mM. In contrast, the two fold activation, with respect to the basal activity, due to 10 µM (-)-adrenaline was inhibited by labetalol in a dose-dependent manner. Inhibition was half maximal with 0.7 µM labetalol and total with 10 µM of inhibitor. Labetalol had the same pattern of action on the adrenaline-stimulated adenylate cyclase of rat heart (data not shown). This result demonstrates that labetalol inhibits adenylate cyclase through the β-adrenoceptor, with no direct effect on the catalytic moiety of the enzyme.

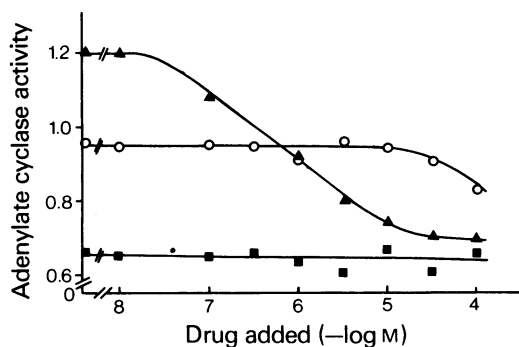


Figure 1 Action of labetalol on rat liver adenylate cyclase activity. Adenylate cyclase activity was tested in the presence of 0.5 mM ATP, 3 mM MgCl_2 , 1 mM EDTA in 50 mM Tris-HCl pH 7.6 and of increasing concentrations of labetalol with no other addition (■), or with 10 μM (-)-adrenaline (▲) or 10 μM GTP (○). Membranes (25 μg protein) were incubated for 10 min at 33°C and the assays were performed as described in the text. Adenylate cyclase activity is expressed as nmol of cyclic AMP formed in 10 min per mg of membrane protein.

Inhibition of adrenaline-stimulated adenylate cyclase: comparison of labetalol with other β -adrenoceptor blocking agents

We compared the action of labetalol and other β -antagonists on the adrenaline-stimulated cyclase activity from rat liver plasma membrane. We have previously demonstrated that the stimulation by catecholamines of liver adenylate cyclase is of the β_2 type (Lacombe, René, Guellaën & Hanoune, 1976). As depicted in Figure 2, adenylate cyclase activity stimulated by 10 μM (-)-adrenaline, was tested in the presence of increasing concentrations (0.1 nM to 0.1 mM) of labetalol, propranolol, butoxamine and practolol. Half-maximal inhibition occurred in the presence of 0.36 μM labetalol, 0.02 μM propranolol, and of 5.35 μM butoxamine, a specific β_2 -antagonist, while the β_1 antagonist, practolol, was almost ineffective up to 0.1 mM. The corresponding apparent K_i estimated from the IC_{50} values, according to Cheng & Prusoff (1973) were: 17, 1 and 250 nM for labetalol, propranolol and butoxamine respectively.

Interaction of labetalol with the binding of [^3H]-dihydroalprenolol to the β -receptors of rat liver plasma membranes

Since we demonstrated that labetalol inhibited the early events following the β -adrenoceptor occupancy by (-)-adrenaline, namely the activation of adenylate cyclase, it was of interest to study the possible interac-

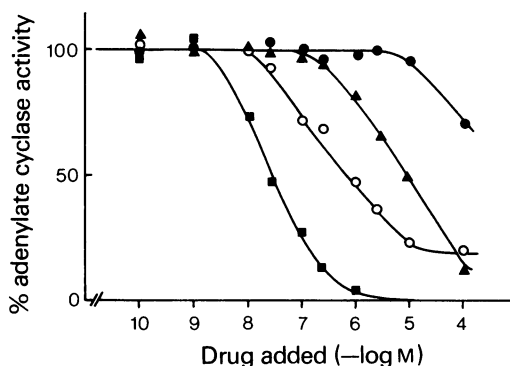


Figure 2 Inhibition of adrenaline-stimulated adenylate cyclase from rat liver plasma membrane by different β -adrenoceptor antagonists. Adenylate cyclase activity was assayed in presence of various amounts of labetalol (○), (-)-propranolol (■), butoxamine (▲) and practolol (●). The amount of protein present per assay was 40 μg . The incubation medium (60 μl) contained 0.5 mM ATP, 3 mM MgCl_2 , 1 mM EDTA, 10 μM (-)-adrenaline in 50 mM Tris-HCl pH 7.6. The effect of the various drugs is expressed as the percentage of the activity with no antagonist added, 100% being 1.6 nmol cyclic AMP formed in 10 min per mg protein.

tion of this drug with the β -adrenoceptors. These sites are now easily characterized by the binding of the specific β -antagonist (-)-[^3H]-dihydroalprenolol (Alexander *et al.*, 1975). Figure 3, shows results of experiments in which various agonists and antagonists competed with 5.4 nM (-)-[^3H]-DHA for binding to the β -adrenoceptors of rat liver plasma membranes. (-)-Adrenaline, (-)-isoprenaline, (-)-propranolol and labetalol were used at final concentrations ranging from 0.1 nM to 1 mM; 50% of the specific [^3H]-DHA bound to rat liver plasma membranes was displaced by 0.025 μM (-)-isoprenaline, (-)propranolol and labetalol, and by 0.5 μM (-)-adrenaline. The apparent K_i s calculated from these values were 12 nM and 145 nM respectively (Table 1). It thus appears that, at least *in vitro*, labetalol acted directly on β -receptors and competed with (-)-[^3H]-DHA for the β -sites with an affinity 10 times greater than that of (-)-adrenaline.

Interaction of labetalol with the binding of [^3H]-dihydroergocryptine to the α -receptor of rat liver plasma membrane

The rat liver possesses both α - and β -adrenoceptors, as confirmed by binding studies. Since labetalol acted directly on β -receptors we decided to investigate whether it also acted directly on the α -receptors *in vitro*. For this purpose, we used [^3H]-DHEC, a

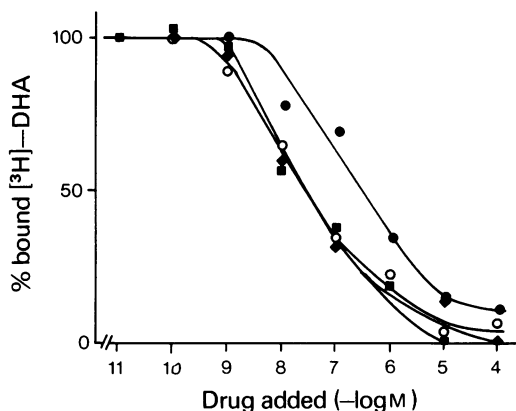


Figure 3 Inhibition of [3 H]-dihydroalprenolol ([3 H]-DHA) binding to rat liver plasma membranes by various drugs. Rat liver plasma membranes (1.2 mg protein/ml) were incubated with 5.4 nM [3 H]-DHA and with increasing concentrations of (-)-adrenaline (●), (-)-isoproterenol (◆), labetalol (○) and (-)-propranolol (■). Binding assays of [3 H]-DHA were carried out as described in the text. Results are expressed as percentage of the amount of tritiated ligand bound in the absence of any drug (30 fmol [3 H]-DHA specifically bound/mg membrane protein). Each value is the mean of triplicate determinations.

specific α -antagonist (Williams *et al.*, 1976) to characterize the α -receptor in rat liver. As shown in Figure 4, labetalol, (-)-adrenaline and (-)-isoprenaline, within the range 10 nM to 1 mM, competed with the binding of 2.7 nM [3 H]-DHEC to rat liver plasma membranes. Labetalol and (-)-adrenaline displaced half of the maximal specific binding at 0.29 μ M and 1.8 μ M respectively. (-)-Isoprenaline was less efficient having an EC_{50} of 120 μ M. The apparent K_{15} were

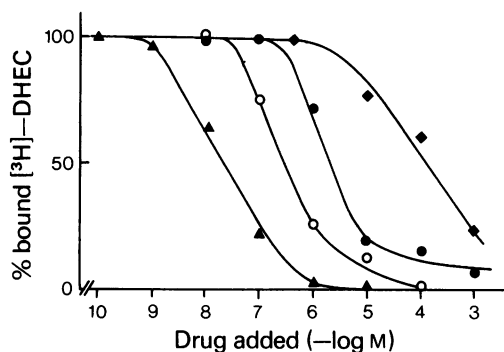


Figure 4 Inhibition of [3 H]-dihydroergocryptine ([3 H]-DHEC) binding to rat liver plasma membrane by various drugs. Rat liver plasma membranes (0.60 mg protein/ml) and 2.7 nM [3 H]-DHEC were incubated with increasing concentrations of (-)-adrenaline (●), (-)-isoprenaline (◆), labetalol (○) and phentolamine (▲). Assays were carried out as described in the text. Results are expressed as percentage of the amount of tritiated ligand bound in the absence of any drug (400 fmol/mg protein). Each value is the mean of triplicate determinations.

0.17, 1.2 and 74 μ M for labetalol, (-)-adrenaline and (-)-isoprenaline respectively (Table 1). These results clearly demonstrated the direct, dual effect of labetalol on both α - and β -adrenoceptors in rat liver plasma membranes.

Effects of labetalol on the binding of [3 H]-dihydroalprenolol to β -adrenoceptors in rat heart membranes

It is now generally admitted that β -adrenoceptors can be separated in β_1 - and β_2 -subtypes (Lands, Luduena & Buzzo, 1967), the β -adrenoceptor being β_1 in the heart (Burges & Blackburn, 1972) and β_2 in the liver

Table 1 Apparent K_{15} of adrenoceptor agonists and antagonists on α - and β -adrenoceptors

	Apparent K_{15} (nM)			
	Liver		Heart	
	β sites	α sites	β sites	α sites
Labetalol	12	170	77	510
(-)-Propranolol	12	14,000	4	8200
Phentolamine	50,000	9.5	22,000	8.2
(-)-Isoprenaline	12	74,000	ND***	ND
(-)-Adrenaline	145	1200	ND	ND
[3 H]-dihydroalprenolol	4.4*	ND	6.2**	ND
[3 H]-dihydroergocryptine	ND	4.5*	ND	4.5**

* personal results, unpublished; ** values obtained by Ciaraldi & Marinetti (1977); ***ND: not determined.

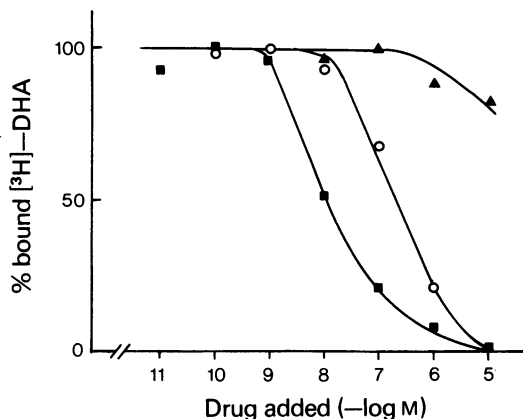


Figure 5 Inhibition of [^3H]-dihydroalprenolol ([^3H]-DHA) binding to rat heart membranes by various drugs. Rat heart membranes (1 mg protein/ml) and 8 nM [^3H]-DHA were incubated with increasing concentrations of (-)-propranolol (■), phentolamine (▲) and labetalol (○). Assays were carried out as described in the text. Results are expressed as percentage of the amount of tritiated ligand bound in the absence of any drug (100 fmol/mg membrane protein). Each value is the mean of triplicate determinations.

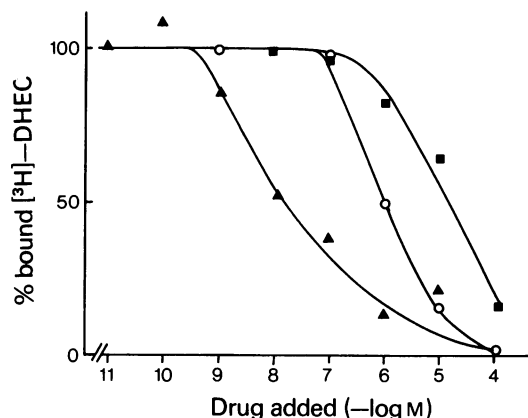


Figure 6 Inhibition of [^3H]-dihydroergocryptine ([^3H]-DHEC) binding to rat heart membranes by various drugs. Rat heart membranes (0.52 mg protein/ml) and 4.5 nM [^3H]-DHEC were incubated with increasing concentrations of (-)-propranolol (■), phentolamine (▲) and labetalol (○). Results are expressed as percentage of the amount of tritiated ligand bound in the absence of any drug (250 fmol/mg membrane protein). Each value is the mean of triplicate determinations.

(Lacombe *et al.*, 1976). We therefore performed similar experiments in rat heart. Figure 5 shows the results of experiments in which (-)-propranolol, labetalol and phentolamine at concentrations ranging from 10 μM to 10 nM , competed with 8 nM [^3H]-DHA for binding to the β -receptors of rat heart plasma membranes. The respective concentrations of (-)-propranolol and labetalol which prevented the occupancy of 50% of the specific binding sites were 11 nM and 210 nM, corresponding to apparent K_i s of 4 and 77 nM. Phentolamine was almost ineffective since 10 μM of this compound diminished the specific binding by only 20%.

Effects of labetalol on the binding of [^3H]-dihydroergocryptine to α -adrenoceptors in rat heart membranes

In parallel with the study of the action of labetalol on the β_1 -receptor in rat heart, we assessed the effect of this drug on the binding of [^3H]-DHEC to the α -adrenoceptor in the same organ. Figure 6 depicts results of an experiment in which 4.5 nM [^3H]-DHEC compared with concentrations of (-)-propranolol, labetalol and phentolamine ranging from 0.1 nM to 0.1 mM. Half-maximal displacement of the specific [^3H]-DHEC bound occurred at 14.5 nM, 1.1 μM and 3.2 μM for phentolamine, labetalol and (-)-propranolol respectively. Corresponding apparent K_i s (Table 1) were 8 nM, 510 nM and 8.2 μM respectively.

Discussion

This paper presents a biochemical demonstration of the action of labetalol as a dual α - and β -antagonist. Labetalol suppressed the stimulation of adenylate cyclase by (-)-adrenaline through β -receptors, with an apparent K_i of 17 nM. It was effective neither on adenylate cyclase activated by GTP nor on basal activity (Figure 1). The experiment depicted in Figure 2 shows that labetalol was 10 fold less potent than propranolol in inhibiting adenylate cyclase. This fact is in good agreement with previous pharmacological observations (Farmer *et al.*, 1972; Brittain & Levy, 1976; Collier, Dawnay, Nachev & Robinson, 1972). Thus labetalol appeared to behave as a β -adrenoceptor antagonist at the membrane level. Further investigations were focused on the direct action of labetalol with the receptor sites. For this study, we took advantage of recent developments in the characterization of adrenoceptors by stereospecific, tritiated ligands, namely [^3H]-DHEC and [^3H]-DHA for the α - and β -receptor sites respectively. According to the literature, this characterization includes the classical criteria of saturability, reversibility and stereospecificity. We have recently extended these results to rat liver plasma membranes (unpublished observations). Labetalol was found to compete with (-)-[^3H]-DHA and [^3H]-DHEC for β - and α -receptor sites with apparent K_i s of 12 nM and 170 nM in liver membranes and

77 nm and 510 nm in heart membranes, respectively (Table 1). In both cases, labetalol was more efficient on β - than on α -receptors. Among the two types of β -receptors, labetalol was 3 times as efficient on β_2 - (liver) as on β_1 - (heart) receptors. The results obtained by binding studies are in good agreement with those previously obtained on the β -receptor adenylate cyclase system. In both cases, the K_1 of labetalol was of the same order of magnitude: 12 to 17 nM.

The results of the biochemical approach used in the present study are in accordance with previous observations, performed *in vivo* (Richards, 1976; Dollery, 1976; Collier *et al.*, 1972). They clearly demon-

strate that labetalol is indeed a dual α - and β -adrenoceptor antagonist which inhibits the binding of catecholamines to their membranous receptor sites. While the knowledge of the site of action of this drug may contribute to the assessment of its pharmacological, toxicological and clinical properties, it may also hopefully lead to a better understanding of the structure-function relationship of α - and β -adrenoceptors.

This work was supported by the Institut National de la Santé et de la Recherche Médicale, the Délégation Générale à la Recherche Scientifique and by the Fondation pour la Recherche Médicale.

References

- ALEXANDER R.W., WILLIAMS L.T. & LEFKOWITZ R.J. (1975). Identification of cardiac β -adrenergic receptors by $(-)[^3\text{H}]$ alprenolol binding. *Proc. Natl Acad. Sci., U.S.A.*, **72**, 1564–1568.
- BRITTAI R.T. & LEVY G.P. (1976). A review of the animal pharmacology of labetalol, a combined α - and β -adrenoceptor blocking drug. *Br. J. clin. Pharmacol.*, **3**, supplement 3, 681–694.
- BROWN E.M., FEDAK S.A., WOODARD C.J., AURBACH G.D. & ROBBARD D. (1976). β -adrenergic receptor interactions. Direct comparison of receptor interaction and biological activity. *J. biol. Chem.*, **251**, 1239–1246.
- BURGES R.A. & BLACKBURN K.J. (1972). Adenylcyclase and the differentiation of β -adrenoceptors. *Nature*, **235**, 249–250.
- CIARALDI T. & MARINETTI G.V. (1977). Thyroxine and propylthiouracil effects *in vivo* on alpha and beta adrenergic receptors in rat heart, *Biochem. biophys. Res. Commun.*, **74**, 984–991.
- CHENG Y. & PRUSOFF W.H. (1973). Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.*, **22**, 3099–3108.
- COLLIER J.G., DAWNAY N.A.H., NACHEV C.H. & ROBINSON B.F. (1972). Clinical investigation of an antagonist at α - and β -adrenoceptors AH 5158A. *Br. J. Pharmacol.*, **44**, 286–293.
- DARGIE H.J., DOLLERY C.T. & DANIEL J. (1976) Labetalol in resistant hypertension. *Br. J. clin. Pharmacol.*, **3**, supplement 3, 751–755.
- DOLLERY C.T. (1976). Closing remarks: current status of labetalol. *Br. J. clin. Pharmacol.*, **3**, supplement 3, 823–824.
- FARMER J.B., KENNEDY I., LEVY G.P. & MARSHALL R.J. (1972). Pharmacology of AH5158; a drug which blocks both α - and β -adrenoceptors. *Br. J. Pharmacol.*, **45**, 660–675.
- HANOUNE J., LACOMBE M.L. & PECKER F. (1975). The epinephrine-sensitive adenylate cyclase of rat liver plasma membranes: role of guanyl nucleotides. *J. biol. Chem.*, **250**, 4569–4574.
- HANOUNE J., STENGEL D., LACOMBE M.L., FELDMANN G. & COUDRIER E. (1977). Proteolytic activation of rat liver adenylate cyclase by a contaminant of crude collagenase from *Clostridium histolyticum*. *J. biol. Chem.*, **252**, 2039–2045.
- KOCH G. (1976a). Combined α - and β -adrenoceptor blockade with oral labetalol in hypertensive patients with reference to haemodynamic effects at rest and during exercise. *Br. J. clin. Pharmacol.*, **3**, supplement 3, 729–732.
- KOCH G. (1976b). Haemodynamic effects of combined α - and β -adrenoceptor blockade after intravenous labetalol in hypertensive patients at rest and during exercise. *Br. J. clin. Pharmacol.*, **3**, supplement 3, 725–728.
- LACOMBE M.L., RENÉ E., GUELLAËN G. & HANOUNE J. (1976). Transformation of the β_2 adrenoceptor in normal rat liver into a β_1 type in Zajdela hepatoma. *Nature*, **262**, 70–72.
- LANDS A.M., LUDUENA F.P. & BUZZO H.J. (1967). Differentiation of receptors responsive to isoproterenol. *Life Sci.*, **6**, 2241–2249.
- LERAY F., CHAMBAUT A.M., PERRENOUD M.L. & HANOUNE J. (1973). Adenylate cyclase activity of rat liver plasma membranes: hormonal stimulations and effect of adrenalectomy. *Eur. J. Biochem.*, **38**, 185–192.
- NEVILLE D.M. (1968). Isolation of an organ specific protein antigen from cell-surface membrane of rat liver. *Biochim. biophys. Acta*, **154**, 540–552.
- RICHARDS D.A. (1976). Pharmacological effects of labetalol in man. *Br. J. clin. Pharmacol.*, **3**, supplement 3, 721–723.
- SCHMITT H. & POCHET R. (1977). *In vivo* labelling of β -adrenergic receptors on rat glioma cells. *Febs Lett.*, **76**, 302–305.
- SPORN J.R. & MOLINOFF P.B. (1976). β -adrenergic receptors in rat brain. *J. Cycl. Nucl. Res.*, **2**, 149–161.
- SUTHERLAND E.W., ØYE I. & BUTCHER R.W. (1965). The action of epinephrine and the role of the adenyl cyclase system in hormone action. *Recent. Prog. Horm. Res.*, **21**, 623–646.
- WHITE A.A. (1974). Separation and purification of cyclic nucleotides by alumina column chromatography. In *Methods in Enzymology* ed Hardmann J.G. & O'Malley B.W. vol. 38c, pp 41–46. New York: Academic Press.
- WILLIAMS L.T., MULLIKIN D. & LEFKOWITZ R.J. (1976). Identification of α -adrenergic receptors in uterine smooth muscle membranes by $[^3\text{H}]$ dihydroergocryptine binding. *J. biol. Chem.*, **251**, 6915–6923.

(Received August 15, 1977.)