MOLECULAR PHARMACOLOGY, 12, 203-207

Inhibition of Isoproterenol Activation of Adenylate Cyclase by Metoprolol, Oxprenolol, and the *para* Isomer of Oxprenolol

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(Received September 12, 1975)

SUMMARY

PETRACK, BARBARA & ANDREW J. CZERNIK (1976) Inhibition of isoproterenol activation of adenylate cyclase by metoprolol, oxprenolol, and the *para* isomer of oxprenolol. *Mol. Pharmacol.*, 12, 203-207.

The *beta* adrenergic blocking agents metoprolol, oxprenolol, and the *para* isomer of oxprenolol were evaluated as inhibitors of isoproterenol activation of adenylate cyclase (EC 4.6.1.1) in membrane preparations from dog heart and liver. Metoprolol, like practolol, was selective, since both drugs inhibited activation of the heart enzyme 10 times more effectively than the liver enzyme. However, metoprolol was considerably more potent than practolol, although it was less active than propranolol. Oxprenolol was a highly effective inhibitor in these systems, comparable in potency to propranolol. Oxprenolol, like propranolol, was a nonspecific *beta* blocker, since these drugs inhibited both heart and liver enzyme systems with the same potency. In contrast, the *para* isomer of oxprenolol was $beta_1$ selective, although it was considerably less potent than oxprenolol. The biochemical data are consistent with the pharmacological effects of these drugs and add further support to the proposed role of the adenylate cyclase system in the molecular mechanisms associated with *beta* adrenergic stimulation and inhibition.

INTRODUCTION

Sutherland and co-workers (1) demonstrated that *beta* adrenergic effects of catecholamines are mediated by cyclic 3',5'-AMP. The mechanism involves an interaction between the catecholamine and its specific *beta* adrenergic receptor on the cell membrane; this interaction activates the catalytic component of the adenylate cyclase system, which is also contained in the membrane. The increased cAMP[1] which is generated within the cell then elicits physiological reactions characteristic of the particular cell or tissue.

Recent evidence indicates that *beta* adrenergic receptors may be divided into at least two distinct groups according to differences in relative affinities of agonists and antagonists (2-6). Thus receptors found in the heart, adipose tissue, and small intestine are among the $beta_1$ type, whereas those in the liver and in the smooth muscle of the bronchioles and blood vessels are among the $beta_2$ type. However, the *beta* adrenergic receptors exhibit relative rather than absolute selectivity.

Further studies demonstrated that the tissue selectivity of *beta* adrenergic receptor-blocking agents, observed in intact pharmacological models, is reflected at the molecular level by similar selectivity as antagonists of catecholamine-stimulated

[1] The abbreviation used is: cAMP, adenosine cyclic 3',5'-monophosphate.

adenylate cyclase. Thus practolol, a $beta_1$-blocking agent in pharmacological studies, also exhibited this selectivity as an inhibitor of catecholamine activation of adenylate cyclase, whereas propranolol appeared to be a nonspecific *beta* blocker in both pharmacological and biochemical models (7–10).

This report summarizes our biochemical studies with some newer *beta* blockers, the pharmacological profiles of which have recently been reported (4, 11, 12). Pharmacological studies indicate that oxprenolol, like propranolol, is a nonspecific *beta* blocker, while metoprolol and the *para* isomer of oxprenolol are selective $beta_1$ antagonists. In our studies the drugs were tested as inhibitors of isoproterenol activation of adenylate cyclase in preparations of dog heart and liver.

MATERIALS AND METHODS

Adenylate cyclase was prepared from dog heart and liver according to the procedure described by Murad (7). A mongrel dog was killed with pentobarbital sodium. A part of the myocardial ventricle and a part of each lobe of the liver were removed, chopped, and washed with cold 0.9% NaCl. Washed particles were prepared from 20 g of each tissue by the following procedure. Tissue was homogenized in 180 ml of 0.25 M sucrose at 4°, first in a Waring Blendor and again in a Potter-Elvehjem ground glass homogenizer. The homogenates were centrifuged at 4300 × *g* for 30 min. Each pellet was washed with 150 ml of cold 0.25 M sucrose, recentrifuged, and then suspended in 0.25 M sucrose, and the volume was adjusted to 40 ml. The preparations were divided into 1.5-ml aliquots, quickly frozen, and stored at −70°. Each aliquot was used in only one experiment, so that the enzyme preparation was never thawed more than once before use. Protein content of the final preparation was measured according to Lowry *et al.* (13).

Adenylate cyclase was assayed essentially as described by Rodbell (14). Each assay mixture (100 μl, final volume) contained membrane particles (equivalent to 150 μg of protein), [α-^{32}P]ATP (1 mM; specific activity, 100 cpm/pmole), $MgCl_2$ (5 mM), creatine phosphate (40 mM), creatine kinase (0.1 mg), EDTA (1 mM), cAMP (4 mM), GTP (0.1 mM), Tris-HCl buffer (50 mM, pH 7.5), isoproterenol (0.5 μM), and various amounts of *beta* blockers.

The tubes were incubated for 10 min at 37°. The reaction was stopped by addition of 0.1 ml of a solution which contained cAMP (13 mM), ATP (40 mM), sodium dodecyl sulfate (1%), and [^{3}H]cAMP (0.08 μCi, to monitor recovery). cAMP was then isolated via Dowex 50 chromatography, followed by two negative adsorptions on nascent $BaSO_4$ as described by Rodbell (14), based on the procedure devised by Krishna *et al.* (15). Each experiment also included blanks in which enzyme was added after the stopping solution, and controls with and without isoproterenol.

RESULTS

The activation of dog heart and liver adenylate cyclase by isoproterenol is shown in Table 1. Isoproterenol was used as the activator because of its superior *beta* agonist properties. A submaximal concentration of isoproterenol (0.5 μM) was employed to allow for greater expression of *beta*-blocking activity. This concentration resulted in approximately 70% of the maximal activation with each enzyme. Even at this submaximal concentration, isoproterenol induced a 3.5-fold activation of heart adenylate cyclase and a 2.2-fold activation of the liver enzyme under our assay conditions.

TABLE 1

Isoproterenol activation of adenylate cyclase of dog heart and liver

Adenylate cyclase was assayed with and without isoproterenol (0.5 mM). The enzyme preparations and the assay are described under MATERIALS AND METHODS. Values are means ± standard errors of two experiments, in which the basal and isoproterenol-activated controls were done in triplicate.

Tissue	cAMP formation	
	No isoproterenol	Plus isoproterenol
	nmoles/mg/10 min	
Heart	0.35 ± 0.01	1.26 ± 0.02
Liver	0.07 ± 0.006	0.16 ± 0.003

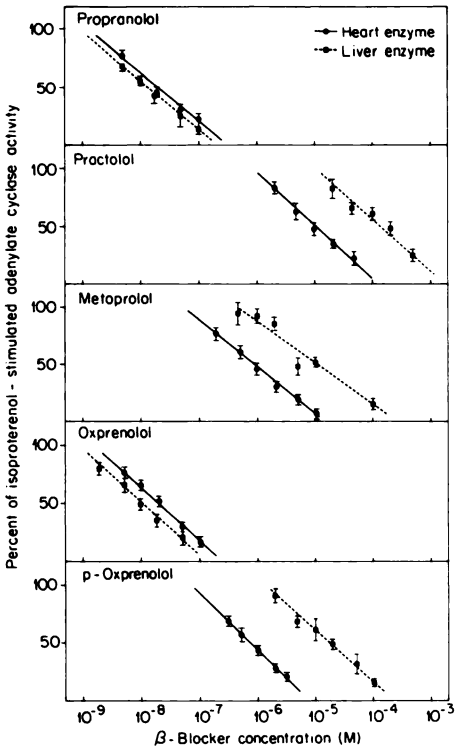


FIG. 1. *Inhibition of isoproterenol activation of adenylate cyclase from dog heart and liver by beta blockers*

The data are expressed as percentage of the isoproterenol activation of adenylate cyclase activity observed in the absence of drug (after subtraction of the basal activity). Each point is the average of duplicate determinations in two separate experiments.

Figure 1 illustrates the inhibition of isoproterenol activation of adenylate cyclase from heart and liver as a function of the concentration of metoprolol, oxprenolol, and the *para* isomer of the latter. Data for propranolol and practolol are included, for comparison. The IC_{50} values taken from these data are summarized in Table 2. The ratio of IC_{50} (liver) to IC_{50} (heart) is shown as an index of relative $beta_1$ specificity. An index of 1.0 suggests equal activity for blockade of both $beta_1$ and $beta_2$ receptors; values greater than unity indicate cardioselectivity.

Oxprenolol was a highly effective inhibitor in these systems, comparable in potency to propranolol. Furthermore, oxprenolol, like propranolol, was a nonspecific *beta* blocker, since these drugs inhibited both heart and liver enzyme systems with approximately the same potency. In contrast, the *para* analogue of oxprenolol inhibited isoproterenol activation of heart adenylate cyclase 25 times more effectively than it inhibited activation of the liver enzyme. However, its potency was considerably less than that of oxprenolol, although it was more than 10 times more potent than practolol. The IC_{50} for metoprolol also was markedly lower with heart than with liver adenylate cyclase, and its potency was similar to that of *p*-oxprenolol.

DISCUSSION

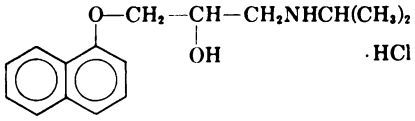
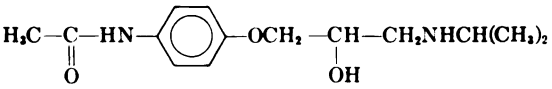
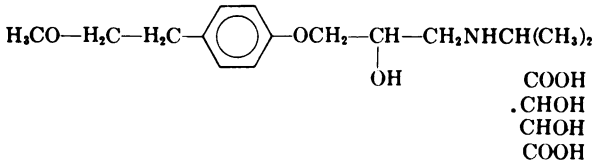
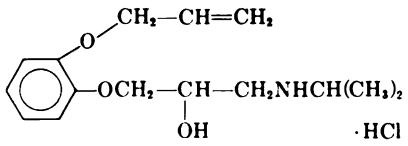
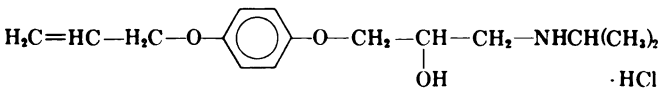
The data in this report show that the *beta* adrenergic blocking agents oxprenolol, its *para* isomer, and metoprolol inhibited the activation of adenylate cyclase by isoproterenol in membrane preparations of dog heart and liver. Oxprenolol was a very potent, nonspecific *beta* blocker, comparable to propranolol. In contrast, metoprolol and the *para* isomer of oxprenolol selectively inhibited activation of heart adenylate cyclase. Thus they are $beta_1$ blockers, like practolol, but are more than 10 times more potent than practolol.

Examination of the structures of these drugs (Table 2) reveals that each of the $beta_1$-blocking agents bears a substituent in the *para* position, whereas the nonspecific *beta* blockers do not. This relationship between cardioselectivity and *para* substitution was particularly strking with the pair oxprenolol and its *para* isomer. Similar observations were reported in pharmacological studies with this pair of isomers (4), with practolol and its *ortho* isomer (5), and with alprenolol and its *para* analogue (16). Pharmacological studies with trimepranol and its analogues led Zakhari (17) to conclude that *para* substitution in the aromatic ring of *N*-isopropylphenoxypropanolamines is of great importance for imparting myocardial selectivity to the molecule. Our data also indicate that *para* substitution appears to be correlated with this selectivity. To our knowledge, no cardioselective *N*-isopropylphenoxypropanolamine *beta* blocker has yet been reported which

TABLE 2

Relative beta$_1$ specificity of some beta adrenergic-blocking agents

Relative *beta*$_1$ specificity is defined as the ratio of IC_{50} (liver) to IC_{50} (heart).

Compound	IC_{50}		Relative *beta*$_1$ specificity
	Heart	Liver	
	μM	*μM*	
Propranolol	0.02	0.01	0.5
			
Practolol	10.0	135.0	13.5
			
Metoprolol	0.85	11.0	12.9
			
Oxprenolol	0.02	0.01	0.5
			
***p*-Oxprenolol**	0.72	18.0	25.0
			

lacks a substituent in the *para* position. However, the substituent itself is also important, as seen by the difference in potency between practolol and metoprolol. Taken together, these observations on the parallelism between pharmacological and biochemical data further support the proposed role of the adenylate cyclase system in the molecular mechanisms associated with *beta* adrenergic stimulation and inhibition.

ACKNOWLEDGMENT

We would like to express our appreciation to Dr. William D. Cash for valuable advice during the course of this work.

REFERENCES

1. Robison, G. A., Butcher, R. W. & Sutherland, E. W. (1971) *Cyclic AMP*, pp. 145–231, Academic Press, New York.
2. Lands, A. M., Arnold, A., McAuliff, J. P., Luduena, F. P. & Brown, T. G., Jr. (1967) *Nature*, 214, 597–598.
3. Lands, A. M., Luduena, F. P. & Buzzo, H. J. (1967) *Life Sci.*, 6, 2241–2249.
4. Brunner, H., Hedwall, P. R. & Meier, M. (1971) *Naunyn-Schmiedebergs Arch. Pharmakol.*, 269, 219–231.
5. Vaughan Williams, E. M., Bagwell, E. E. & Singh, B. N. (1973) *Cardiovasc. Res.*, 7, 226–240.
6. Weinstock, M., Schechter, Y., Erez, M. & Shtacher, G. (1974) *Eur. J. Pharmacol.*, 26, 191–197.

7. Murad, F. (1973) *Biochim. Biophys. Acta*, **304**, 181–187.
8. Burges, R. A. & Blackburn, K. J. (1971) *Nat. New Biol.*, **235**, 249–250.
9. Augstein, J., Cox, D. A., Ham, A. L., Leeming, P. R. & Snarey, M. (1973) *J. Med. Chem.*, **16**, 1245–1251.
10. Lefkowitz, R. (1975) *Biochem. Pharmacol.*, **24**, 583–590.
11. Brunner, H., Hedwall, P. R., Maier, R. & Meier, M. (1970) *Postgrad. Med. J.*, Nov. suppl., 5–14.
12. Åblad, B., Borg, K. O., Carlsson, E., Ek, L., Johnsson, G., Malmfors, T. & Regårdh, C.-G. (1975) *Acta Pharmacol. Toxicol.*, **36**, Suppl. 5, 7–23.
13. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.*, **193**, 265–275.
14. Rodbell, M. (1972) in *Methods in Cyclic Nucleotide Research* (Chasin, M., ed.), pp. 101–124, Marcel Dekker, New York.
15. Krishna, G., Weiss, B. & Brodie, B. B. (1968) *J. Pharmacol. Exp. Ther.*, **163**, 379–385.
16. Åblad, B., Brogård, M., Carlsson, E. & Ek, L. (1970) *Eur. J. Pharmacol.*, **13**, 59–64.
17. Zakhari, S. (1974) *Eur. J. Pharmacol.*, **29**, 22–29.