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FM_{24}: A LONG LASTING BLOCKER OF RAT LIVER β-ADRENORECEPTORS

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Abstract—The compound FM_{24}, 1-(2-exo-bicyclo[2,2,1]hept-2-ylphenoxy)-3-[(1-methyl-ethyl)amino]-2-propanol, hydrochloride, inhibited isoproterenol-dependent adenylate cyclase from rat liver plasma membranes, while it had no effect on the basal, fluoride-, Gpp(NH)p-, and glucagon-dependent cyclase activities. FM_{24} also blocked the specific binding of (−)[^{3}H]dihydroalprenolol to the rat liver β-adrenoreceptors. The inhibition was of the non-competitive type and could not be reversed by extensive washing, unlike the competitive, reversible, inhibition caused by propranolol. Preincubation with either propranolol or isoproterenol protected the binding sites and adenylate cyclase activity against inactivation by FM_{24}. These findings suggest that FM_{24} behaves as a non-dissociable or a very slowly reversible β-receptor antagonist. This may be due to the hydrophobic nature of the molecule, and is supported by experiments done with several structural analogs of FM_{24}.

Just recently, a new compound, FM_{24}, has been shown to be a long lasting β-adrenergic blocker *in vivo* [1]. In addition to its possible therapeutic action in hypertensions [2], such a compound could be of interest for labeling β-adrenergic receptors from a biochemical point of view. Indeed, a bromoacetylated propranolol analog was shown to bind covalently to specific β-adrenergic receptors [3, 4] and the radiolabeled derivative has been used for characterization of the β-antagonist–receptor complex at the membrane level [5]. However, such an affinity labeling does not allow pharmacological studies of the β-receptors in their native form. On the other hand, noncovalently, tightly bound ligands are of great interest in characterizing the hormonal binding sites, since the possibility does exist of modifying their dissociation kinetics by a non-denaturing process, i.e. temperature variations. As an example, labeling β-receptors with such a ligand would make it possible to assess the pharmacological properties of the receptors during the course of their solubilization and purification. In the present study, we report the *in vitro* apparently irreversible inactivation of β-receptors from rat liver plasma membranes by FM_{24}.

MATERIALS AND METHODS

Materials

(±)Alprenolol (Ciba-Geigy) and (−)propranolol (ICI) were obtained as gifts; (−)isoproterenol and ATP were purchased from Sigma. Creatine kinase, GTP and guanyl-5′-yl imidodiphosphate (Gpp(NH)p) were obtained from Boehringer-Mannheim, and creatine phosphate from Calbiochem. All other reagents were obtained from Merck (Darmstadt) and were of analytical grade. [α-^{32}P]ATP (6–14 Ci/m-mole) was obtained from Amersham Radiochemical Centre, cyclic [8-^{3}H]AMP (13 Ci/m-mole) was from the CEA (Saclay, France) and (−)[^{3}H]dihydroalprenolol was from New England Nuclear Corp.

Methods

Preparation of purified liver plasma membranes. Plasma membranes were obtained from livers of female, adrenalectomized [6], Wistar rats (about 100 g body weight), and prepared according to the procedure devised by Neville [7] up to step 11. The purified membrane preparations were suspended in 1 mM $NaHCO_3$ and stored in liquid nitrogen until use. Protein was measured according to Lowry's procedure, using bovine serum albumin as standard.

Adenylate cyclase assay. Adenylate cyclase [ATP pyrophosphatase lyase (cyclizing) (EC 4.6.1.1)] activity was measured as previously reported [8, 9]. Unless otherwise indicated, the assay medium contained 0.5 mM [α-^{32}P]ATP (10^6 c.p.m.), 3 mM $MgCl_2$, 1 mM EDTA, 1 mM [^{3}H]cyclic AMP ($2 < 10^4$ c.p.m.), 10 μM GTP, 50 mM Tris–HCl (pH 7.6), an ATP regenerating system (25 mM phosphocreatine and creatine phosphokinase, 1 mg/ml) and 20–60 μg of protein in a final volume of 60 μl. Incubation was initiated by the addition of membranes and performed for 5 or 10 min at 30° in a shaking water bath. Reactions were terminated by a modification of the procedure of White [10] as described earlier [9]. Enzymatic activities are expressed as nmoles cyclic AMP formed in 5 or 10 min per mg membrane protein. The results obtained from triplicate determinations agreed within ±5 per cent.

Binding assay. Binding of (−)[^{3}H]dihydroalprenolol was carried out as described previously [11, 12]. 5 nM (−)[^{3}H]Dihydroalprenolol and liver plasma membranes (0.4–0.6 mg protein/ml) were incubated with constant shaking for 10 min at 30° in the complete medium used for adenylate cyclase determination and in a final volume of 400 μl. At the end of the incubation, triplicate aliquots (100 μl) were diluted with 4 ml ice-cold 50 mM Tris–HCl (pH 7.6), and filtered through Whatman GF/C glass fiber filters (24 mm diameter). The filters were then washed with 8 ml ice-cold buffer, dried

and counted in 10 ml ACS aqueous scintillation mixture (Amersham Searle) at an efficiency of 40%. Non-specific binding is defined as binding which is not displaced by 5 μM (±) alprenolol. Specific binding is defined as total radioactivity bound minus nonspecific binding. Results are expressed as fmoles (−)[^{3}H]dihydroalprenolol specifically bound per mg protein.

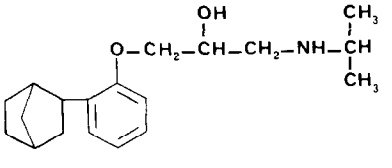
Preincubation experiments. Unless otherwise specified, preliminary incubations of the membranes (1.5–2 mg protein/ml) with β-adrenergic analogs were performed for 15 min at 20° with constant shaking. Membranes were then either used directly for the adenylate cyclase and binding assays, or washed first as follows: the incubation mixture was diluted with 10 vol. ice-cold 50 mM Tris–HCl (pH 7.6) and centrifuged at 2000 *g* for 5 min at 4°. The pellet was resuspended and similarly washed 3 or 4 times. The final pellet was resuspended in buffer for direct use in the adenylate cyclase and binding assays. The recovery of protein after such washing was about 50–60 per cent.

RESULTS

FM_{24} is a specific inhibitor of isoproterenol-stimulated adenylate cyclase. FM_{24}, 1-(2-exo-bicyclo-[2,2,1]hept-2-ylphenoxy)-3-[(1-methyl-ethyl)amino]-2-propanol, hydrochloride (Fig. 1) comprises the side arm of classical β-adrenergic antagonists and a norbornyl group at the ortho position of the phenyl ring.

As shown in Fig. 2, the isoproterenol-stimulated adenylate cyclase activity from rat liver was inhibited by low concentrations of FM_{24}, i.e. 10 nM, maximal inhibition being attained at 1 μM. In addition, the inhibition curve was not modified by the presence of 10 μM GTP in the incubation medium. Therefore this inhibition was specific for isoproterenol-stimulated adenylate cyclase since basal, sodium fluoride-, Gpp(NH)p-, and glucagon-stimulated adenylate cyclase activities were not affected by FM_{24} concentrations up to 10 μM.

Non-competitive inhibition of FM_{24}. The inhibitory effect of FM_{24} was studied next as a function of increasing concentrations of isoproterenol. This was done after a 30 min preincubation of the liver membranes with 50 nM FM_{24}. The effect of propranolol was assayed in a similar manner and the data are depicted in Fig. 3. The addition of 50 nM FM_{24} resulted in a decrease (65 per cent) of the isoproterenol maximal activation, with no change in the apparent affinity of (−)isoproterenol for the enzyme (0.8 μM). This apparently non-competitive inhibition contrasted with the inhibition brought about by 50 nM propranolol, which was of the competitive type. The apparent affinity of (−)propranolol for the system was calculated from this curve according to Furchgott [13], and found to be 7.7 nM.



FM 24

Fig. 1. Structure of FM_{24}.

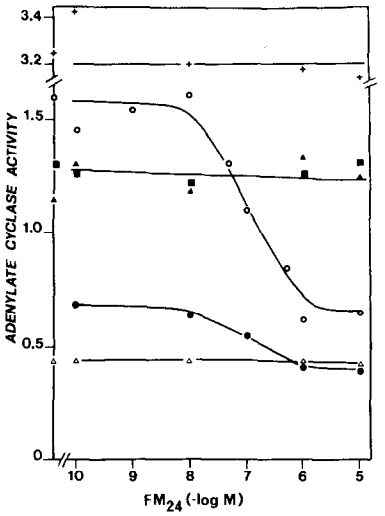


Fig. 2. Specificity of adenylate cyclase inhibition by FM_{24}. Rat liver plasma membranes (0.5 mg protein/ml) were incubated in the presence of the indicated concentrations of FM_{24} for 10 min at 30°. Adenylate cyclase activity was assayed as described under Methods. Results are expressed as nmoles cyclic AMP formed in 10 min per mg protein. Basal activity (△) and 0.1 μM glucagon (+), 10 mM NaF (■), 10 μM Gpp(NH)p (▲), 5 μM (−)isoproterenol (●) and 10 μM GTP plus 5 μM (−)isoproterenol (○) stimulated adenylate cyclase activities are represented. Each point represents the mean of triplicate determinations, agreeing within ±5 per cent.

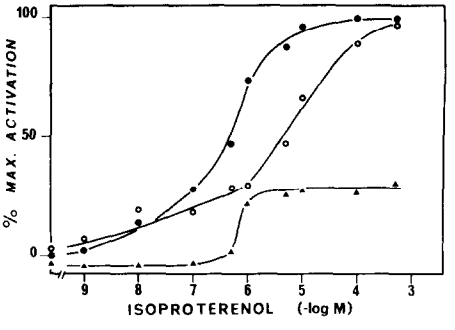


Fig. 3. Comparative inhibition of isoproterenol-stimulated adenylate cyclase by propranolol and FM_{24}. Rat liver plasma membranes (1.5 mg protein/ml) were preincubated for 30 min at 20°, in the absence (●) or in the presence of 50 nM (−)propranolol (○) or FM_{24} (▲), as described under Methods. Membranes were then used directly, without washing, in the (−)isoproterenol-sensitive adenylate cyclase assay. Results are expressed as per cent of maximal activation. Basal and maximal activations of adenylate cyclase by (−)isoproterenol from control membranes were 0.29 and 0.42 nmoles cyclic AMP formed in 10 min/mg protein. Each point represents the mean of triplicate determinations made on two separate experiments with different membrane preparations. It should be noted that, due to a non-specific inactivation of the enzyme during the prolonged time of preincubation of the membranes, isopreterenol-stimulated activities were lower in this experiment than these reported in Fig. 2, in which no preincubation was carried out.

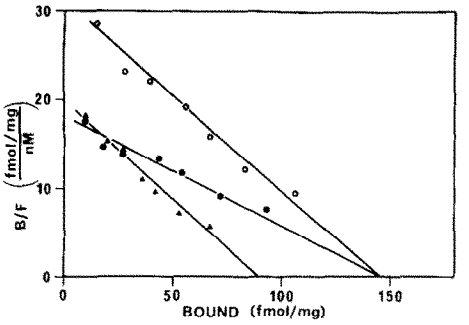


Fig. 4. Scatchard plot of (−)[^{3}H]dihydroalprenolol binding in the presence of β-adrenergic antagonists. Rat liver plasma membranes (1.3 mg protein/ml) were preincubated for 15 min at 20°, with no addition (○), 5 nM (−)propranolol (●) or 10 nM FM_{24} (▲). Membranes were then used directly, without washing in the (−)[^{3}H]dihydroalprenolol binding assay designed for Scatchard analysis, over a range (from 1 to 20 nM) of (−)[^{3}H]dihydroalprenolol concentrations. Each value is the mean of triplicate determinations and data reported are representative of three such experiments, made with different membrane preparations.

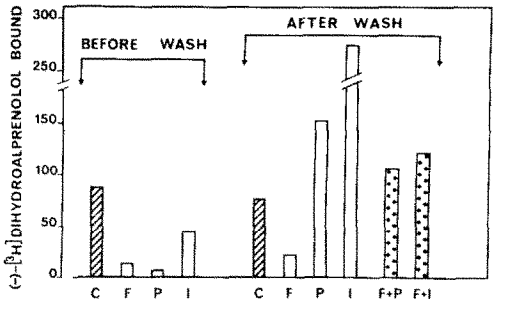


Fig. 6. Effects of isoproterenol, propranolol and FM_{24} on specific binding of (−)[^{3}H]dihydroalprenolol. Rat liver plasma membranes (1.5 mg protein/ml) were preincubated for 30 min at 20°, with no addition (C), 10 μM (−)isoproterenol (I), 1 μM (−)propranolol (P), 0.1 μM FM_{24} (F) or 0.1 μM FM_{24} plus either 1 μM (−)propranolol (F + P) or 10 μM (−)isoproterenol (F + I). Membranes were then washed 5 times in 10 vol. 50 mM Tris–HCl (pH 7.6), as described under Methods. Specific binding of (−)[^{3}H]dihydroalprenolol was assayed before and after washing. Results are expressed as fmoles of (−)[^{3}H]dihydroalprenolol bound per mg of membrane protein. Each value is the mean of triplicate determinations, agreeing within ±5 per cent. These results are representative of three such experiments, made with different membrane preparations.

In further experiments, rat liver plasma membranes were exposed to either 10 nM FM_{24} or 5 nM propranolol and then assayed directly (without washing) for binding activity over a range (from 1 to 20 nM) of (−)[^{3}H]dihydroalprenolol concentrations (Fig. 4). The total number of β-receptors in control membranes was about 145 fmoles/mg protein and the K_D value for (−)[^{3}H]dihydroalprenolol was 4.8 nM. Exposure to 5 nM propranolol resulted in a reduction of the apparent affinity (from 4.8 to 7.7 nM) for the tritiated ligand, with no significant reduction in the total number of binding sites. By contrast, exposure to 10 nM FM_{24} led to a 40 per cent reduction in the number of β-adrenergic sites (from 145 to 90 fmoles mg protein), while the affinity of the unreacted sites for the tritiated ligand remained unchanged. From these data, the equilibrium dissociation constant of (−)propranolol could be calculated using the equation detailed by Furchgott [13]. The value obtained by this method (8.3 nM) was in good agreement with the value (7.7 nM) determined above.

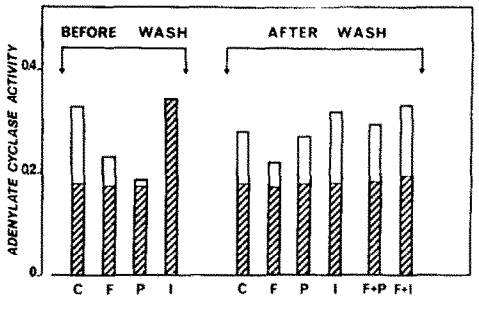


Fig. 5. Effects of isoproterenol, propranolol and FM_{24} on adenylate cyclase activity. Rat liver plasma membranes (1.4 mg protein/ml) were preincubated for 15 min at 20°, with no addition (C), 0.5 μM (−)isoproterenol (I), 50 nM (−)propranolol (P), 5 nM FM_{24} (F), or 5 nM FM_{24} plus either 50 nM (−)propranolol (F + P) or 0.5 μM (−)isoproterenol (F + I). Membranes were then washed 3 times in 10 vol. 50 mM Tris–HCl (pH 7.6) as described under Methods. Basal activities (hatched bars) and 5 μM (−)isoproterenol-stimulated adenylate cyclase activities (open bars) were assayed before and after washing. Each value, expressed as nmoles cyclic AMP formed in 5 min per mg protein, represents the mean of triplicate determinations, agreeing within ±5 per cent. The experiment has been done twice with two different membrane preparations.

Washing experiments. After exposure of rat liver plasma membranes to either 5 nM FM_{24}, 50 nM propranolol, or 0.5 μM isoproterenol for 15 min, followed by three cycles of washing (*cf.* Methods), it appeared that the inhibitory effect of FM_{24} upon the isoproterenol-stimulated adenylate cyclase activity was entirely preserved under conditions in which the effect of isoproterenol and propranolol were completely reversed (Fig. 5).

In another series of experiments, we tested the (−)[^{3}H]dihydroalprenolol binding capacity of rat liver plasma membranes which had been preincubated for 30 min with either 0.1 μM FM_{24}, 1 μM propranolol or 10 μM isoproterenol and washed 5 times afterwards (Fig. 6). Again, FM_{24} appeared to be an apparently irreversible blocker of the β-adrenergic receptors, while isoproterenol and propranolol were completely reversible by washing. In this typical experiment, the (−)[^{3}H]dihydroalprenolol binding capacity of the membranes was significantly enhanced after preincubation of the membranes with either propranolol or isoproterenol, i.e. 150 and 260 per cent over basal capacity of control membranes, respectively.

In these experiments, exposure of the membranes to FM_{24} in the presence of a 10- or 100-fold excess of propranolol (F + P) or isoproterenol (F + I), respectively, resulted in the full protection of adenylate cyclase sensitivity (Fig. 5) and (−)[^{3}H]dihydroalprenolol binding capacity (Fig. 6), as compared to control membranes. Thus, the presence of a reversible ligand in the preincubation step prevented the apparently irreversible inactivation of β-receptors by FM_{24}.

Time-course of the effect of FM_{24}. The ability of

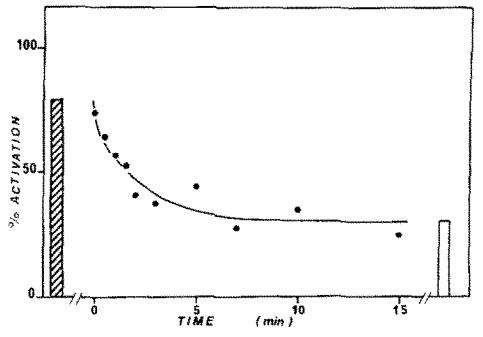


Fig. 7. Time course of the inactivation by FM_{24} of adenylate cyclase. Rat liver plasma membranes (1.6 mg protein/ml) were preincubated with 50 nM FM_{24} for 15 min at 20°. At the indicated times after the beginning of the preincubation, 0.5 μM (−)propranolol was added. After the completion of the 15 min preincubation, membranes were washed 4 times in 10 vol. 50 mM Tris–HCl (pH 7.6). Basal and 5 μM (−)isoproterenol-stimulated adenylate cyclase activities were then assayed as described under Methods. Results are expressed as per cent activation over basal level, which was unchanged in each assay; 0.30–0.33 nmole cyclic AMP formed in 5 min per mg protein. The abcissa represents the time of exposure of the membranes to FM_{24} before the propranolol addition. The bars represent values obtained in samples in which membranes were preincubated for 15 min with either the Tris buffer (hatched bar) or 50 nM FM_{24} only (open bar). In this typical experiment, each value is the mean of triplicate determinations, agreeing within ±5 per cent.

Table 1. Comparative effects of FM_{24} analogs on adenylate cyclase activity

Preincubation of plasma membrane with:	Per cent change in adenylate cyclase activity over basal level	
	Before wash	After wash
No addition	88.5	86.7
FM_{24}	8.1	27.8
Compound I	3.7	11.0
Compound II	16.8	6.3
Compound III	16.8	2.9
Compound IV	−3.5	24.0
Compound V	0.2	−0.2
Compound VI	38.7	84.2

Rat liver plasma membranes (2.3 mg protein/ml) were preincubated for 15 min at 20°, with no addition (C) or 1 μM of either FM_{24} (F), FM_{24} 'Endo' form (I), compound II, compound III, compound IV, compound V or compound VI. Membranes were then washed 5 times in 10 vol. 50 mM Tris–HCl (pH 7.6), as described under Methods. Basal and 5 μM (−)isoproterenol-stimulated adenylate cyclase activities were assayed before and after washing. Each value, expressed as per cent activation over basal level (0.32 nmole cyclic AMP formed in 5 min/mg protein), represents the mean of triplicate determinations, agreeing within ±5 per cent. This experiment was done twice with two different membrane preparations.

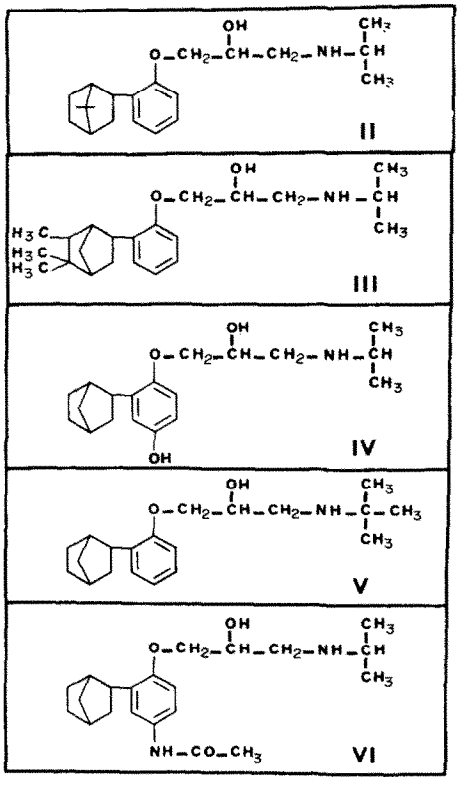


Fig. 8. Structure of the analogs of FM_{24}.

propranolol to protect the binding sites against blockade by FM_{24} was used to estimate the time-course of inactivation of the isoproterenol-stimulated adenylate cyclase. Rat liver plasma membranes were exposed to 50 nM FM_{24} for 15 min at 20°. At the indicated intervals after initiation of the incubation, a sufficient amount of propranolol (0·5 μM) was added to protect the sites not yet inhibited by FM_{24}, and incubation was allowed to continue until a total of 15 min of exposure to FM_{24} was completed. Membranes were then washed and the remaining adenylate cyclase activity was assayed (Fig. 7). The kinetics of β-receptor inactivation by FM_{24} could then be assessed: the expected inactivation was obtained rapidly with an apparent half-time of 72 sec. The maximal inactivating effect was reached by 5 min of incubation, but could not be increased by further incubation. The extent of the maximal inactivation by 50 nM FM_{24} of isoproterenol-dependent adenylate cyclase was about 55 per cent, as compared to the zero time value.

Structural analogs of FM_{24}. We have tested the sensitivity of the adenylate cyclase to various analogs of FM_{24}, the structures of which are represented in Fig. 8. In these experiments, rat liver plasma membranes were preincubated for 15 min at 20° with each analog at 1 μM concentration, then washed 5 times as described under Methods. Basal and isoproterenol stimulated adenylate cyclase activities were then tested. As shown in Table 1, compounds which are methylated either on the side arm or on the norbonyl group behaved as apparently irreversible inhibitors of adenylate cyclase (compounds II, III and V). In contrast, compounds which are substituted by a hydrophilic group behaved as reversible (compound VI) or partially reversible (compound IV) inhibitors.

DISCUSSION

Our results demonstrate the non-equilibrium nature of β-receptors' inactivation by FM_{24} in rat liver plasma membranes, in contrast to the reversible, competitive blockade produced by propranolol (Figs. 3 and 4).

We have previously shown [14] that *in vivo* administration of FM_{24} to rats led to a persistent non-competitive inhibition of isoproterenol-stimulated adenylate cyclase activity in the heart. In contrast, *in vivo* administration of propranolol did not influence the maximal *in vivo* responsiveness of the heart cyclase to isoproterenol. We also demonstrated [14] that the long lasting *in vivo* effect of FM_{24} could not be due to its conversion to an active metabolite.

FM_{24} behaved as a tightly bound blocker of both isoproterenol-dependent adenylate cyclase activity (Fig. 5) and β-adrenergic receptor sites (Fig. 6) in rat liver plasma membranes. Moreover, the presence of isoproterenol or propranolol during exposure of membranes to FM_{24} prevented the inactivation of β-receptors as well as of isoproterenol-stimulated adenylate cyclase. This, and also the fact that FM_{24} did not inhibit the other cyclase activities tested (Fig. 2), strongly suggest that the drug interacts with the same specific β-adrenergic receptor binding sites with which agonist and antagonist drugs act.

The mechanism of irreversible inactivation of β- and α-receptor binding sites by *N*-[2-hydroxy-3-(1-naphtoxy)-propyl]-*N'*-bromo-acetyl-ethylenediamine [3, 4] and phenoxybenzamine [15], respectively, probably involves covalent bond formation by alkylation of a sulfhydryl group. Unlike these compounds, FM_{24} is not halogenated and its binding to β-adrenergic sites probably does not involve covalent binding. Thus, the mechanism of action of FM_{24} may be compared to that of other slowly dissociable, non-covalently bound ligands, i.e. the post-synaptic receptor blocker α-toxin [16], whose half-time of dissociation is 60 hr at room temperature. The structure of FM_{24} (Fig. 1) suggests that its long lasting inactivation of β_2-receptors from rat liver plasma membranes (Figs. 5 and 6) and β_1-receptors from cardiac particulate fractions [14] is due to the rigid, bulky, hydrophobic norbonyl group of the molecule. This is in agreement with the results depicted in Table 1, in which more hydrophobic analogs of FM_{24}, i.e. compounds II, III and V, behaved as irreversible blockers of isoproterenol-dependent adenylate cyclase activity, whereas less hydrophobic compounds, i.e. compound VI, behaved as reversible inhibitors of this activity.

In the experiment reported in Fig. 7, the time-course of β-receptors inactivation by FM_{24} was indirectly assessed and the half-time of inactivation was found to be about 1 min. However, complete inactivation of isoproterenol-dependent adenylate cyclase activity could not be achieved after 15 min of rat liver plasma membranes preincubation with FM_{24}. Several hypotheses could explain these data:

(i) β-Adrenergic receptors could comprise two classes of sites which would bind FM_{24} differently: the binding of FM_{24} to one of them could be slowly dissociable, and the binding to the other could be quickly reversible. However, this is not compatible with the fact that we found only one class of non allosteric β-adrenergic binding sites, both in rat liver (Fig. 4) and rat heart [14], using a labeled reversible antagonist as ligand.

(ii) The local concentration of FM_{24} in the vicinity of β-adrenergic sites could be insufficient to inactivate all the sites, due to binding of the drug to other membrane components, or to the fact that the FM_{24} preparation contained four stereoisomers. Indeed, due to the hydrophobic nature of the FM_{24} molecule, its long lasting effect might involve, in addition to its specific binding to β-receptors, binding to other membrane sites, possibly located in the lipid phase of the membrane.

In addition to results concerning the apparently irreversible action of FM_{24}, we present here data (Figs. 5 and 6) that reveal a 'supersensitivity' of β-adrenergic receptors when rat liver plasma membranes have been preincubated in the presence of either agonist or antagonist reversible ligands, prior to washing. This 'supersensitivity' was not due to differences in protein concentration of washed membranes (about 0.4 mg protein/ml in each sample), but seemed to be time- and ligand concentration-dependent; after 30 min of membranes preincubation with either 10 μM isoproterenol or 1 μM propranolol (Fig. 6), the (−)[^{3}H]dihydroalprenolol binding capacity was dramatically enhanced by 260 and 150 per cent, respectively. These values were less when preincubation time was reduced to 15 min or when the concentration of ligands was decreased (data not shown). It has been already reported that *in vivo* administration of propranolol to rats [17] or preincubation of intact turkey reticulocytes in the presence of (±)propranolol [18] resulted in a 100 per cent increase in the number of β-receptors in heart particulate fraction and on the cell surface, respectively. The fact that we observed such a supersensitivity phenomenon *in vitro*, using isolated plasma membranes, may lead to a better understanding of its mechanism.

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REFERENCES

1. M. Mestre, G. Le Fur and A. Uzan, *Seventh International Congress of Pharmacology, IUPHAR*, 16–21 *July* 1978, Paris, Abstr. 2912, p. 961.
2. O. B. Holland and N. M. Kaplan, *N. Engl. J. Med.* **294**, 930 (1976).
3. D. Atlas and A. Levitzki, *Biochem. biophys. Res. Commun.* **69**, 397 (1976).
4. D. Atlas, M. L. Steer and A. Levitzki, *Proc. natn. Acad. Sci., U.S.A.* **73**, 1921 (1976).
5. D. Atlas and A. Levitzki, *Nature, Lond.* **272**, 370 (1978).
6. F. Leray, A.-M. Chambaut, M.-L. Perrenoud and J. Hanoune, *Eur. J. Biochem.* **38**, 185 (1973).
7. D. M. Neville, *Biochim. biophys. Acta* **154**, 540 (1968).
8. J. Hanoune, M.-L. Lacombe and F. Pecker, *J. biol. Chem.* **250**, 4569 (1975).
9. J. Hanoune, D. Stengel, M.-L. Lacombe, G. Feldmann and E. Coudrier, *J. biol. Chem.* **252**, 2039 (1977).
10. A. A. White, in *Methods in Enzymology* (Eds. J. G. Hardmann and B. W. O'Malley) Vol. 38C, p. 41, Academic Press, New York (1974).
11. G. Guellaën, M. Yates-Aggerbeck, G. Vauquelin, D. Strosberg and J. Hanoune, *J. biol. Chem.* **253**, 1114 (1978).

12. M. Aggerbeck, G. Guellaën and J. Hanoune, *Br. J. Pharmac.* **62**, 543 (1978).
13. R. F. Furchgott, *Ann. N.Y. Acad. Sci.* **139**, 553 (1967).
14. G. Le Fur, P.-H. Schmelck, P. Geynet, J. Hanoune and A. Uzan, *Life Sci.* **23**, 1841 (1978).
15. L. T. Williams and R. J. Lefkowitz, *Molec. Pharmac.* **13**, 304 (1977).
16. M. Weber and J.-P. Changeux, *Molec. Pharmac.* **10**, 1 (1974).
17. G. Glaubiger and R. Lefkowitz, *Biochem. biophys. Res. Commun.* **78**, 720 (1977).
18. E. Hanski and A. Levitzki, *Life Sci.* **22**, 53 (1978).