Biochemical Pharmacology, Vol. 35, No. 9, pp. 1511–1516, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

CRITICAL ASSESSMENT OF THE PLATELET ADENYLATE CYCLASE SYSTEM AS A POTENTIAL MODEL FOR TESTING ALPHA$_2$ ADRENERGIC ACTIVITY

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(*Received* 22 *July* 1985; *accepted* 5 *November* 1985)

Abstract—We have studied the effects of KUM 32 and CBS 1276, two clonidine-related drugs, upon the adenylate cyclase system of human platelets. Both drugs behaved as potent antagonists of epinephrine-induced platelet aggregation. [^{3}H]Yohimbine binding studies revealed that the drugs bind to the alpha$_2$ adrenergic receptor of human platelets. KUM 32 and CBS 1276 also behaved as strong inhibitors of adenylate cyclase activity. This inhibition, which was not competitive with respect to ATP, is not an alpha$_2$ adrenergic phenomenon since it was not antagonized by yohimbine and was still observed in the absence of GTP. Moreover, pretreatment of platelet membranes with islet activating protein from *Bordetella pertussis* (IAP) had no effect on the inhibition by KUM 32, CBS 1276 and adenosine, although it completely reversed the effect of epinephrine and partially reversed the effect of clonidine. These results show that clonidine-like drugs may have different impacts on the adenylate cyclase system of human platelets. This system cannot be used as a pharmacological predictive test for alpha$_2$ adrenergic agonist activity, as various compounds, known to have central alpha$_2$ adrenergic agonist properties, do not behave as full agonists for the alpha$_2$ adrenergic receptor of human platelets.

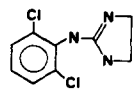
During the past few years, alpha adrenergic receptors have been divided in two different subtypes, alpha$_1$ and alpha$_2$, according to their relative affinities for various agonists and antagonists [1]. Tritiated ligand binding studies have allowed the determination of the subtype of alpha adrenergic receptors in numerous organs [2, 3]. In the brain, which possesses both alpha$_1$ and alpha$_2$ subtypes, it has been shown that the activation of alpha$_2$ adrenergic receptors led to a decrease in the arterial blood pressure [4]. This hypotensive effect has triggered the synthesis of numerous molecules, structurally related to the alpha$_2$ adrenergic agonist clonidine. Although the physiological effect of central alpha$_2$ adrenergic receptors (i.e. hypotension) has been extensively studied, nothing is known concerning the biochemical events which follow the binding of agonists to the brain receptor and promote the physiological response. However, the biochemistry of alpha$_2$ adrenergic responses has been subject to many studies in peripheral organs such as human platelets. Human platelets possess alpha adrenergic receptors which are exclusively of the alpha$_2$ subtype [5]. The activation of this receptor triggers a GTP-dependent decrease in the adenylate cyclase activity, mediated through a regulatory protein usually called Ni [6–8]. These events are accompanied by the aggregation of the platelets, which represents the physiological response of platelets to adrenergic stimuli [9].

The aim of the present report is to study the relationship between adenylate cyclase inhibition and alpha$_2$ adrenergic activity for two analogs of clonidine (Fig. 1): CBS 1276, an hydrophilic derivative developed for glaucoma therapy [10] and KUM 32, known to have central hypotensive property [11].

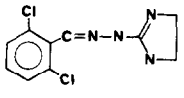
MATERIALS AND METHODS

Materials

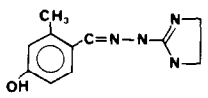
Islet activating protein (IAP) was purchased from List Biological Laboratories Inc. (Campbell, U.S.A.). ATP, creatine phosphokinase, phosphocreatine, cyclic AMP, GTP, (−)epinephrine, adenosine and thymidine were obtained from Sigma. [^{3}H]-Yohimbine was from New England Nuclear Co. (Boston, MA). [^{3}H]cyclic AMP was from the CEA (Saclay, France). (Alpha ^{32}P-ATP) was from the Radiochemical Centre (Amersham, U.K.). Clonidine (Boehringer Ingelheim, Reims, France), KUM 32 (Sandoz, Rueil, France), CBS 1276 (Chauvin Blache, Montpellier, France), oxymetazoline (Farmex, Courbevoie, France), naphazoline (Ciba,



clonidine



KUM 32



CBS 1276

Fig. 1. Chemical structure of clonidine, KUM 32 and CBS 1276.

Rueil, France), guanfacine (Sandoz), BHT 920 (Thomae, F.R.G.), guanoxabenz and yohimbine (Roussel-Uclaf, Paris, France) were obtained as gifts. All other reagents were from standard commercial sources.

Methods

Platelet membranes. Human platelets were obtained from the Centre Départemental de Transfusion Sanguine, Hôpital Henri Mondor, France, or from the Centre de Transfusion Sanguine, Montpellier, France. Platelet membranes were prepared as previously described [12], except that the membranes were washed and resuspended in 50 mM Tris–HCl with no addition of Mg^{2+}.

IAP treatment of human platelet membranes. IAP was stored at a concentration of 0.1 mg/ml, at 4°, in 0.1 M Na^+-phosphate buffer pH 7.6 containing 0.5 M Na^+. IAP or vehicle (0.1 ml) was added to platelet membranes (0.5–1 mg) and incubated for 30 min at 30° in a final volume of 0.5 ml containing 2 mM ATP, 20 mM DTT, 0.1 mM GTP, 3 mM NAD, 10 mM thymidine and 50 mM Tris–HCl, pH 7.5. At the end of the incubation, platelet membranes were washed twice in 10 ml of 50 mM Tris–HCl, pH 7.5 for 10 min at 40,000 *g*, and finally resuspended in the same buffer and used for the adenylate cyclase assay.

Binding and adenylate cyclase assays. These were conducted as previously described [12].

Platelet aggregation. Venous blood was drawn from healthy young male volunteers who had taken no drugs for two weeks, in 60 ml plastic syringe containing 6 ml of 3.8% Na^+ citrate. Platelet-rich plasma (PRP) was prepared by centrifugation for 15 min at 500 *g* at room temperature. The extent of platelet aggregation was determined optically with a Labintec aggregometer HU 176. Aliquots (0.5 ml) of PRP were prewarmed at 37° before addition of aggregating agents.

RESULTS

Binding experiments

Tritiated yohimbine is a highly specific ligand of human platelets $alpha_2$ adrenergic receptors [13]. The affinity of clonidine, KUM 32 and CBS 1276 for this receptor was measured by displacing specific [^{3}H]yohimbine binding with increasing concentrations of each drug (Fig. 2). The EC_{50} values obtained were as follows: yohimbine (0.01 μM), KUM 32 (0.2 μM), clonidine (0.22 μM), epinephrine (0.3 μM), CBS 1276 (15 μM). These results clearly demonstrate that epinephrine, clonidine, KUM 32 and CBS 1276 can interact with the same $alpha_2$ adrenergic receptor.

These results are not consistent with previous physiological and pharmacological studies of the central effect of clonidine-related drugs. Timmermans *et al.* [14] have shown that in rat brain membranes labeled with [^{3}H]clonidine, KUM 32 displayed a 10 times lower affinity than clonidine. This discrepancy can be explained both by the difference of the ligand used (yohimbine in our experiments vs clonidine) and the preparation studied (human platelets vs rat brain).

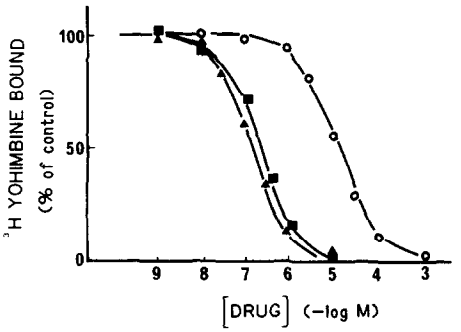


Fig. 2. Binding of KUM 32, CBS 1276 and clonidine to human platelet membranes $alpha_2$ adrenergic receptors. Human platelet membranes (0.2 mg proteins) were incubated for 30 min at 25° in the presence of [^{3}H]yohimbine (8 μM) and various concentrations of each drug as described in Methods. Results are expressed as the percent of specific [^{3}H]yohimbine binding in the absence of drug. Specific binding is calculated as the difference of [^{3}H]yohimbine bound in the absence or presence of 10 μM cold yohimbine. Each point is the mean of triplicate determinations. Clonidine (■), KUM 32 (▲), CBS 1276 (○).

Platelet aggregation experiments

Epinephrine, acting via an $alpha_2$ adrenergic receptor, triggers the aggregation of human platelets, or potentiates the aggregation induced by other agents such as ADP [9, 15]. KUM 32 and CBS 1276 were unable to induce aggregation even at a concentration of 0.1 mM. They, however, antagonized the aggregating potency of epinephrine (Fig. 3). Moreover, as shown in Fig. 4, although epinephrine and clonidine were able to increase the extent of aggregation induced by a low dose of ADP as previously described [15, 16], this effect was not shared by KUM 32 and CBS 1276. We also found that KUM 32 and CBS 1276 were able to antagonize the potentiating effect of epinephrine on ADP-induced aggregation. These experiments clearly show that KUM 32 and CBS 1276 behave as antagonists at the $alpha_2$ adrenergic receptor of human platelets and do not possess the partial agonist pro-aggregating property of clonidine.

Adenylate cyclase experiments

It is now largely demonstrated that $alpha_2$ adrenergic activation in human platelets promotes a decrease in the activity of adenylate cyclase either in basal conditions or after stimulation with PGE_1 or forskolin [12, 17]. Figure 5 shows that KUM 32 and CBS 1276 are strong inhibitors of basal adenylate cyclase activity, in contrast with clonidine which, as previously described [15], only slightly decreases enzyme activity. The order of potency obtained for the inhibition of cyclase: KUM 32 > CBS 1276 > clonidine does not correlate with the binding studies. Moreover, the inhibitory effect of the drugs is the same when GTP is absent and is not antagonized by yohimbine (not shown), although $alpha_2$ adrenergic processes are known to be GTP-dependent [18] and yohimbine-sensitive. We, therefore, investigated the process of the adenylate cyclase inhibition by these drugs in order to ascertain whether this process was related to a competition with ATP. Figure 6A shows that KUM 32 and CBS 1276 were able to reduce

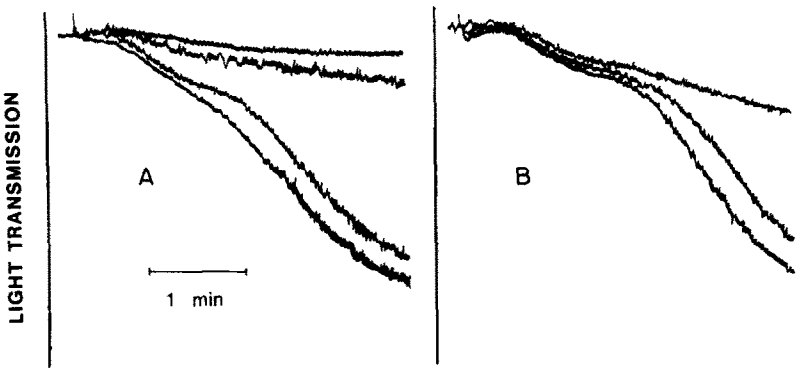


Fig. 3. Effect of KUM 32 and CBS 1276 on the epinephrine-induced aggregation of human platelets. Platelet aggregation was monitored as described in Materials and Methods in the presence of epinephrine 100 μM as aggregating agent. (A) Without or with prior addition of KUM 32: 10, 20 and 100 μM. (B) Without or with prior addition of CBS 1276: 10 and 100 μM.

the velocity of the forskolin-stimulated activity of adenylate cyclase with little or no modification of the apparent affinity of the enzyme for the substrate, MgATP. An Eadie–Hofstee plot of the results (Fig. 6B) demonstrated the non-competitive effect of the drugs: the V_{max} in the absence of inhibitors was 363 pmole min^{-1} mg^{-1} and fell to 155 and 193 pmole min^{-1} mg^{-1} in the presence of 1 mM KUM 32 and CBS 1276 respectively. The apparent affinity for ATP was not significantly modified: Apparent K_m was 0.082 mM in the absence of drugs and 0.043 and 0.059 mM in the presence of KUM 32 and CBS 1276, respectively.

Pretreatment with islet-activating protein

Islet-activating protein (IAP) is a toxin extracted from *Bordetella pertussis*, and selectively blocks the GTP-sensitive Ni protein by transferring an ADP ribose moiety from NAD to the 41 K subunit of Ni [19]. It therefore prevents the inhibitory effect of compounds, such as epinephrine, which inhibit adenylate cyclase activity via Ni and does not interfere with adenosine, a non-competitive inhibitor of adenylate cyclase, which is known to act via a GTP-independent pathway: the P site [20, 21]. It was thus tempting to hypothesize that the inhibitory effect of clonidine, KUM 32 and CBS 1276 were mediated through a "P site-like" mechanism. We therefore investigated the effect of a preincubation in the presence of IAP on the inhibition of adenylate cyclase by epinephrine, clonidine, KUM 32 and CBS 1276. Figure 7 shows that IAP pretreatment completely

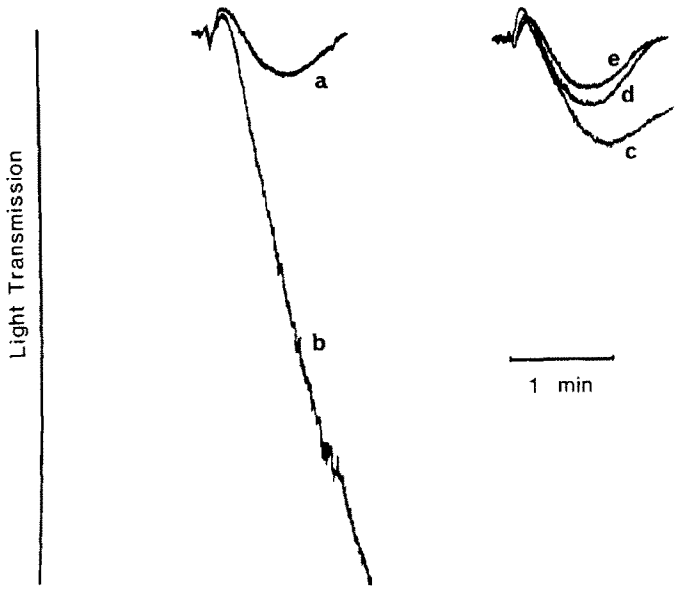


Fig. 4. Proaggregating effect of epinephrine, KUM 32 and CBS 1276 and clonidine on the aggregation induced by ADP. Platelet aggregation was monitored as described in Materials and Methods using ADP 1 μM as aggregating agent: (a) ADP 1 μM alone; (b) ADP with the prior addition of epinephrine 100 μM; (c) clonidine 10 mM added before ADP; (d) KUM 32 100 mM added before ADP; (e) CBS 1276 100 mM added before ADP.

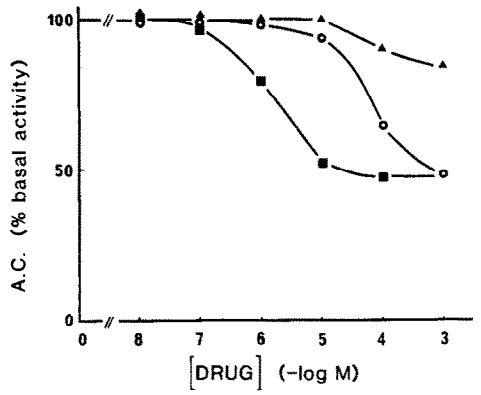


Fig. 5. Dose–response pattern for the inhibition of human platelet adenylate cyclase by clonidine, KUM 32 and CBS 1276. Human platelet membranes were incubated for 10 min at 30° with increasing concentrations of clonidine (▲), KUM 32 (■), CBS 1276 (○), and assayed for adenylate cyclase activity as described in Methods. Each point is the mean of triplicate determinations, and is expressed as the percent of basal activity measured in the absence of drug.

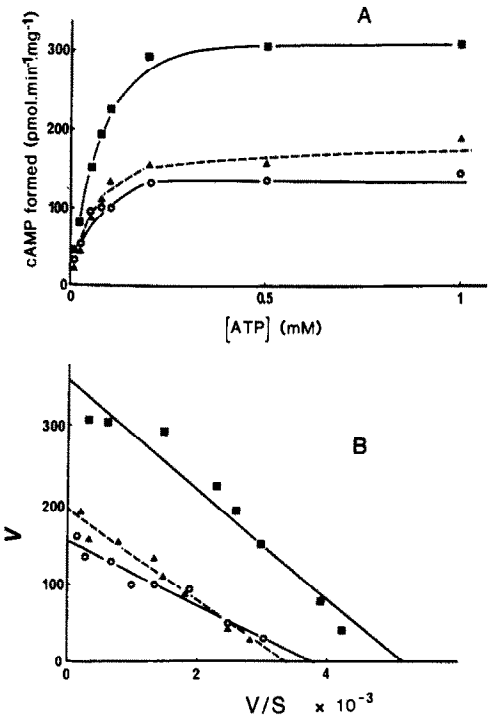


Fig. 6. Kinetic study of inhibition of human platelet adenylate cyclase by KUM 32 and CBS 1276. (A) Adenylate cyclase activity was assayed as described in Methods, with increasing concentrations of ATP, in the presence of a constant amount of [^{32}P]ATP (1.2×10^6 cpm), Mg^{2+} (5 mM) and forskolin (0.5 μM). Basal: ■, KUM 32 (1 mM) ○, CBS 1276 (1 mM) ▲. Each point is the mean of triplicate determination. (B) Eadie–Hofstee plot of the data shown in panel (A). Each point represents the velocity of the reaction (V expressed as pmole cAMP min^{-1} mg^{-1}) plotted as a function of V/S where S is the concentration of ATP (in mM). Lines were obtained from linear regression analysis: ■ basal, ○ KUM 32, ▲ CBS 1276. For all curves: $r = 0.99$, $P < 0.001$.

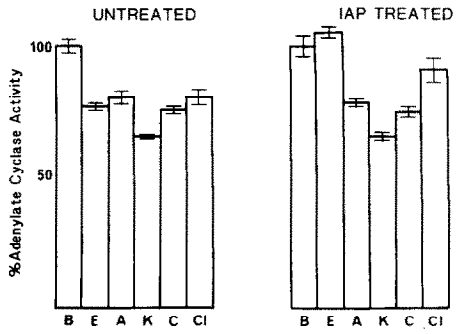


Fig. 7. Effect of IAP treatment on the inhibition of adenylate cyclase. Human platelet membranes were pretreated with or without IAP as described in Methods. Adenylate cyclase activity was assayed in the presence of forskolin (1 μM) as described in Methods. B, Basal; E, with epinephrine (1 mM) and GTP (0.1 mM); A, with adenosine (0.1 mM); K, with KUM 32 (1 mM); C, with CBS 1276 (1 mM); Cl, with clonidine (1 mM). Each result is the mean of triplicate determinations ± S.E.M.

abolishes the GTP-dependent epinephrine inhibition of adenylate cyclase activity, as we had already described [22]. On the contrary, we observed no modification of the inhibition triggered by KUM 32, CBS 1276 or adenosine. The data obtained for clonidine are inconclusive, since a small inhibition persisted after IAP pretreatment. In any case, these results strongly suggest that the potent inhibition of adenylate cyclase observed with KUM 32 and CBS 1276 occurs through a pathway independent of Ni, in a manner similar to the P site-related adenosine action.

Other alpha$_2$ adrenergic agents

Our results clearly demonstrate that KUM 32 and CBS 1276 do not behave as alpha$_2$ adrenergic agonists in human platelets. The question therefore arises as to whether these results are only due to the molecular structure of the two compounds, or is an intrinsic characteristic of the receptor itself. To answer that question, we have studied the effects of various alpha$_2$ adrenergic agonists and antagonist on both the alpha$_2$ adrenergic-mediated inhibition of adenylate cyclase, and the platelet aggregation in the presence of epinephrine. The central alpha$_2$ adrenergic agonist potencies of oxymetazoline, BHT 920, KUM 32, naphazoline, clonidine and guanabenz (structurally close to guanoxabenz) have recently been assessed and compared [11]. Guanfacine is also a central alpha$_2$ adrenergic agonist drug [23], and yohimbine is a powerful antagonist of central alpha$_2$ adrenergic-mediated effects. The results obtained are summarized in Table 1. None of the drugs were able to induce platelet aggregation. On the contrary, they were able to antagonize the aggregation induced by epinephrine with an order of potencies: yohimbine > naphazoline = oxymetazoline > KUM 32 > guanfacine > CBS 1276 > guanoxabenz. This order is different from the one obtained by Timmermans *et al.* at the central level [11]. Adenylate cyclase experiments allow the distinction of two classes of drugs: (i) KUM 32, CBS 1276 and guanoxabenz,

Table 1. Effects of various alpha$_2$ adrenergic drugs on the epinephrine-induced aggregation and adenylate cyclase activity

Drug concentration (μM)	Inhibition of platelet aggregation (%)			Inhibition of adenylate cyclase (%)		
	10	50	100	10	100	1000
KUM 32	60	70	90	20	48	53
CBS 1276	29	58	85	2	10	35
Guanoxabenz	29	67	77	0	10	30
Guanfacine	40	85	94		No inhibition	
Naphazoline	91	100	100		—	
Oxymetazoline	94	100	100		—	
BHT 920		n.d.			—	
Yohimbine	100	100	100		—	

Experiments were performed as described in Methods. The inhibition of aggregation was measured using epinephrine (50 μM) as aggregating agent. n.d. = not determined.

which are strong inhibitors of adenylate cyclase (we verified that guanoxabenz inhibition was not reversed by IAP, and not GTP-dependent, indicating a mechanism similar to the one of KUM 32 and CBS 1276); (ii) oxymetazoline, guanfacine, BHT 920, naphazoline and yohimbine, a second class of drugs which have no effect on the activity of adenylate cyclase. They, however, reversed the inhibition of adenylate cyclase induced by epinephrine.

These data clearly demonstrate that central alpha$_2$ adrenergic agonists behave as antagonists at the alpha$_2$ adrenergic receptors of human platelets, and suggest that the two alpha$_2$ receptors (i.e. central and platelet) are of clearly different types.

DISCUSSION

In the present report, we demonstrate that two clonidine-related drugs, KUM 32 and CBS 1276, interact at two different levels in the adenylate cyclase system of human platelets.

The compounds bind to the alpha$_2$ adrenergic receptor with an order of potency: clonidine = KUM 32 > CBS 1276. Aggregation experiments show that the drugs behave as epinephrine antagonists, although clonidine displays a partial agonist activity for the aggregation induced by ADP, as previously reported [15].

KUM 32, CBS 1276 and, to a lesser extent, clonidine are able to inhibit the activity of adenylate cyclase. This inhibitory effect, which is not competitive with respect to ATP, does not appear to be related to an alpha$_2$ adrenergic pathway for the following reasons: (i) the order of potency of the drugs in inhibiting the adenylate cyclase: KUM 32 > CBS 1276 > clonidine is markedly different from the order of affinity at the alpha$_2$ adrenergic receptor; (ii) the inhibition of adenylate cyclase does not require GTP and is not reversed by yohimbine, two properties which are opposite to the characteristics of the alpha$_2$-adrenergic mediated inhibition of adenylate cyclase; (iii) pretreatment of the membranes with islet-activating protein (IAP) completely abolishes the epinephrine-mediated inhibition of adenylate cyclase, but does not alter the effect of KUM 32 and CBS 1276. These properties of KUM 32 and CBS 1276 are also shared by another hypotensive drug, guanoxabenz. Most of the alpha$_2$ adrenergic hypotensive drugs tested behave, on the contrary, as antagonists of the effects of epinephrine on the platelets, both on aggregation and adenylate cyclase inhibition.

The present findings support two different conclusions:

(1) The dual effects of KUM 32, CBS 1276 and guanoxabenz can be explained by an interaction with two different "receptors". The drugs behave as antagonists of the alpha$_2$ adrenergic receptors, but are potent agonists at another inhibitory site of the adenylate cyclase. This second site seems to be analogous to the P site of adenosine, which is known to be independent of the Ni protein [21]. These results are also in agreement with the previous concept developed by different authors [24, 25] that the interaction of epinephrine with the adenylate cyclase system is not directly responsible for its aggregating action in human platelets. Along the same line, Motulsky *et al.* [26] recently demonstrated that epinephrine preincubation of human platelets appears to desensitize alpha$_2$ adrenergic-mediated platelet aggregation whereas inhibition of adenylate cyclase system is not desensitized under the same circumstances.

(2) Our present findings also support the view that two different types of alpha$_2$ adrenergic receptors could exist. Drugs which behave as agonist at the central level are potent antagonists of the human platelet alpha$_2$ adrenergic receptors. This extends previous results which showed that guanfacine and guanabenz were potent antagonists of the epinephrine-induced aggregation [27]. In the same way, clonidine and oxymetazoline have been previously shown to be very weak agonists for the alpha$_2$ adrenergic inhibition of platelet adenylate cyclase with an intrinsic activity ranging from 0.09 to 0.14 for oxymetazoline and of 0.52 for clonidine, when compared to epinephrine [28, 29]. It, therefore, appears that binding experiments with tritiated ligands are not sufficient to characterize fully a specific type of receptor. Although tritiated yohimbine binds to central and platelet alpha$_2$ adrenergic receptors, it is likely that these two types of receptors are different

entities; and, when testing the alpha$_2$ adrenergic property of a molecule, one should be very careful in the choice of the model in which the experiments will be performed. The pharmacological behaviour of one drug at the alpha$_2$ adrenergic receptor can be different from one tissue to another. A similar subclassification has been proposed by various authors for the alpha$_1$ adrenergic receptor [14, 30].

Inhibition of adenylate cyclase is clearly not sufficient to demonstrate an agonist behavior via the platelet alpha$_2$ adrenergic receptor, unless this inhibition is GTP-dependent through a Ni subunit.

Acknowledgements—We wish to thank Drs S. Adnot and B. Vargaftig for their helpful and valuable discussion of this work, and L. Rosario and V. Brouteland for their expert secretarial assistance. This work was supported by the Fondation pour la Recherche Médicale, the Institut National de Sante et de la Recherche Medicale and the Université Paris–Val de Marne.

REFERENCES

1. S. Berthelsen and W. A. Pettinger, *Life Sci.* **21**, 595 (1977).
2. B. B. Hoffman and R. J. Lefkowitz, *Biochem. Pharmac.* **29**, 452 (1980).
3. B. B. Hoffman, A. De Lean, C. L. Wood, D. D. Schocken and R. J. Lefkowitz, *Life Sci.* **24**, 1739 (1979).
4. P. B. M. W. M. Timmermans, A. M. C. Schoop, H. Y. Kwa and P. A. Van Zwieten, *Eur. J. Pharmac.* **70**, 7 (1981).
5. B. B. Hoffman, D. Mullikin-Kilpatrick and R. J. Lefkowitz, *J. biol. Chem.* **255**, 4645 (1980).
6. E. W. Salzman and L. L. Neri, *Nature* **224**, 609 (1969).
7. K. H. Jakobs, W. Saur and G. Schultz, *Fedn Eur. Biochem. Soc. Lett.* **85**, 167 (1978).
8. T. Katada, G. M. Bokoch, J. K. Northup, M. Ui and A. G. Gilman, *J. biol. Chem.* **259**, 3568 (1984).
9. J. R. O'Brien, *Nature* **200**, 4908 (1963).
10. E. Battais, J. P. Conduzorgues, C. Bonne, D. Sincholle and C. Coquelet, *Drugs of the Future* **9**, 175 (1984).
11. P. B. M. W. M. Timmermans, A. de Jonge, J. C. A. Van Meel, F. P. Slothorst-Grisdijk, E. Lam and P. A. Van Zwieten, *J. med. Chem.* **24**, 502 (1981).
12. P. A. Insel, D. Stengel, N. Ferry and J. Hanoune, *J. biol. Chem.* **257**, 7485 (1982).
13. H. J. Motulsky, S. J. Shattil and P. A. Insel, *Biochem. biophys. Res. Commun.* **97**, 1562 (1980).
14. P. B. M. W. M. Timmermans, A. de Jonge, M. J. M. C. Thoolen, B. Wilffert, H. Batink, and P. A. Van Zwieten, *J. med. Chem.* **27**, 495 (1984).
15. D. C. Stump and D. E. MacFarlane, *J. Lab. clin. Med.* **102**, 779 (1983).
16. J. A. Grant and M. C. Scrutton, *Nature* **277**, 659 (1979).
17. J. Moskowitz, J. P. Harwood, W. D. Reid and G. Krishna, *Biochim. biophys. Acta* **230**, 279 (1971).
18. N. Ferry, S. Adnot, A. Borsodi, M. L. Lacombe, G. Guellaen and J. Hanoune, *Biochem. biophys. Res. Commun.* **108**, 708 (1982).
19. T. Katada and M. Ui, *Proc. natn Acad. Sci. U.S.A.* **79**, 3129 (1982).
20. C. Londos and J. Wolff, *Proc. natn Acad. Sci. U.S.A.* **74**, 5482 (1977).
21. J. Premont, G. Guillon and J. Bockaert, *Biochem. biophys. Res. Commun.* **90**, 513 (1979).
22. M. Goodhardt, N. Ferry, M. Buscaglia, E. E. Baulieu and J. Hanoune, *EMBO J.* **3**, 2653 (1984).
23. C. T. Dollery and P. Jerie, *J. clin. Pharmac.* **10**, (Suppl. 1) (1980).
24. K. A. Clare, M. C. Scrutton and N. T. Thompson, *Br. J. Pharmac.* **82**, 467 (1984).
25. T. M. Connolly and L. E. Limbird, *J. biol. Chem.* **258**, 3907 (1983).
26. H. Motulsky, S. J. Shattil, N. Ferry, D. Rozansky and P. A. Insel, *Molec. Pharmac.* (in press).
27. E. Glusa and F. Markwardt, *Biochem. Pharmac.* **30**, 1359 (1981).
28. B. S. Tsai and R. J. Lefkowitz, *Molec. Pharmac.* **16**, 61 (1979).
29. B. B. Hoffman, T. Michel, T. B. Brenneman and R. J. Lefkowitz, *Endocrinology* **110**, 926 (1982).
30. J. C. McGrath, *Biochem. Pharmac.* **31**, 467 (1982).