Biochemical Pharmacology, Vol. 29, pp. 643–645.
© Pergamon Press Ltd. 1980. Printed in Great Britain.
0006-2952/80/0215–0643 $02.00/0

SHORT COMMUNICATIONS

Adrenergic receptor of the alpha$_1$-subtype mediates the activation of the glycogen phosphorylase in normal rat liver

(*Received* 10 *August* 1979; *accepted* 1 *October* 1979)

For rat liver plasma membranes, tritiated dihydroergocryptine has been used as a label for the alpha-adrenergic receptor [1, 2]. That tritiated DHEC* does indeed label the alpha-adrenergic receptor is supported by the fact that its binding is displaced by various drugs with an order of potency typical of an alpha-adrenoreceptor [1, 2] and by the direct correlation between the interaction of alpha-adrenergic agonists or antagonists with tritiated DHEC binding sites of rat liver plasma membranes and the stimulation (or suppression) of glycogen phosphorylase (EC 2.4.1.1. 1,4-alpha-D-glucan: orthophosphate alpha-glucosyl transferase) activity in isolated hepatocytes, an alpha-receptor mediated effect [3–5]. Alpha-adrenergic receptors have been subdivided, on an anatomic basis, into two subtypes, alpha$_1$ and alpha$_2$, referring, respectively, to post- and pre-synaptic sites [6–8]. However, for alpha-adrenergic receptors found in tissues lacking synapses, subdivision on an anatomic basis may be inappropriate [9–11]. In such tissues, alpha-adrenergic receptors may be more usefully subdivided as to their alpha$_1$ or alpha$_2$ subtype on the basis of their pharmacologic characteristics [10, 11]. The goal of the present study was to elucidate the pharmacologic subtype, alpha$_1$ or alpha$_2$, of the rat liver alpha-adrenoreceptor which was defined by using tritiated DHEC binding in plasma membranes or by the activation of glycogen phosphorylase in isolated hepatocytes.

Rat liver plasma membranes were purified as previously described [12]. Rat hepatocytes were isolated from male Wistar rats (230–330 g body wt) according to the procedure of Seglen [13] with the modifications introduced by Le Cam *et al.* [14]. The specific binding of tritiated dihydroergocryptine to the purified plasma membranes was measured as previously described [15]. The glycogen phosphorylase a activity was assayed according to Hue *et al.* [16], except that [^{14}C]-glucose-1-phosphate was used at a final concentration of 15 mM (0.01 μCi/μmole). After incubation at 30° for 20 min, the reaction was stopped according to the method of Gilboe *et al.* [17]. Protein concentration was measured by the procedure of Lowry [18], using bovine serum albumin as standard. K_D and K_i values were calculated according to Cheng and Prusoff [19]. K_a values represented the concentration of each agonist causing half the maximal activation of the enzyme by the same agent [9, 10]. [^{3}H]dihydroergocryptine (24 Ci/mmole) and alpha-D[U-

* The abbreviations used were: DHEC dihydroergocryptine; K_D: dissociation constant; K_i: inhibition constant; K_a: activation constant.

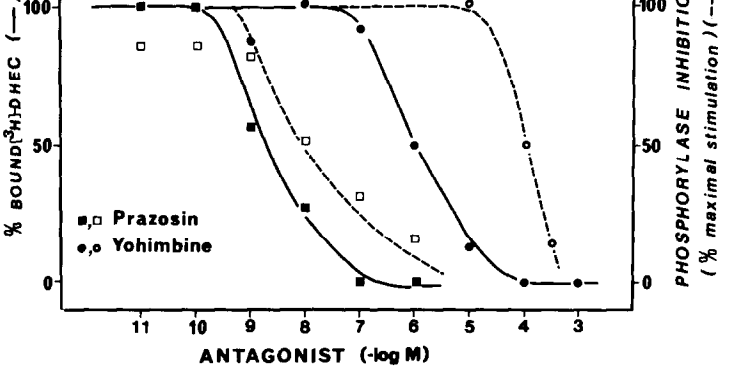

Fig. 1. Inhibition of tritiated DHEC binding to rat liver plasma membranes and inhibition of the (−)epinephrine-stimulated glycogen phosphorylase activity of rat hepatocytes by alpha adrenergic antagonists. Plasma membranes (0.5 mg protein/ml) were incubated at 30° for 10 min with 9 nM DHEC and increasing concentrations of prazosin (■) or yohimbine (●). Results are expressed as per cent of the amount of tritiated DHEC specifically bound in the absence of any antagonist (1100 fmoles DHEC/mg protein). Each point is the mean of a triplicate determination. Isolated hepatocytes (49 × 10^6 cells in 5.8 ml) were preincubated with 4 g/l glucose at 37° for 40 min. Aliquots (125 μl) of cells were incubated with increasing concentrations of prazosin (□), yohimbine (○) or with buffer, for 6 min at 37°. (−)Epinephrine (5 μM final concentration) or buffer were then added. The incubation at 37° was stopped 1 min later by freezing the tubes in a dry ice–acetone mixture. Phosphorylase a was assayed as described earlier. Basal level and maximal activity of the enzyme were, respectively, 0.12 and 0.24 μmole of glucose-1-phosphate transformed in 20 min per mg of protein. The enzyme activity is expressed as per cent of the maximal response over basal level. Each point is the mean of a quadruplicate determination which agreed within 5 per cent.

Table 1. Interaction of alpha-adrenergic agonists and antagonists with tritiated DHEC binding sites of plasma membranes and with glycogen phosphorylase in hepatocytes*

	[^{3}H]-DHEC binding site	Glycogen phosphorylase	
	K_D (μM)	K_i (μM)	K_a (μM)
Antagonists			
Prazosin	0.00047	0.00085	
Phenoxybenzamine	0.01300	0.30000	
Yohimbine	0.33000	11.00000	
Agonists			
(−)phenylephrine	2.0		1.0
Methoxamine	10.0		160.0
(±)normetanephrine	66.0		210.0
Mixed Agonists–Antagonists			
Clonidine	0.30	10.0	200.0
Naphazoline	0.25	6.9	n.d.†
Oxymetazoline	1.00	13.0	22.0

* K_D, K_i and K_a values were determined as described earlier and were usually the average of two or three determinations which agreed within 5 per cent.
† n.d.: not determined

^{14}C] glucose-1-phosphate (0.3 Ci/mmole) were purchased, respectively, from New England Nuclear Co. and from the Radiochemical Centre (Amersham). Prazosin (Pfizer), yohimbine (Roussel-Uclaf), phentolamine, naphazoline (Ciba-Geigy), methoxamine (Burroughs), phenoxybenzamine (Smith, Kline & French Laboratories), clonidine (Boehringer Ingelheim), oxymetazoline (Farmex) were obtained as gifts; (−)phenylephrine, (±)normetanephrine, (−)epinephrine, glycogen type II from oyster, alpha-D-glucose-1-phosphate grade 1, hepes, glycylglycine (Sigma), crude collagenase (CLS type) (Worthington) were from the chemical sources indicated. All other chemicals were from Merck (Darmstadt).

Prazosin and yohimbine, two alpha-adrenergic antagonists known to bind preferentially to alpha$_1$ and alpha$_2$ receptors, respectively [7, 20], were tested as competitors with DHEC for its binding sites. The competition curves are depicted in Fig. 1. Prazosin (K_D = 470 pM) appeared to be 700-fold more potent than yohimbine (K_D = 0.33 μM) in displacing tritiated DHEC from the alpha-adrenoreceptors in purified plasma membranes (Table 1). Moreover, we observed that both drugs displaced tritiated DHEC in a monophasic manner. These results showed that there was one class of sites, alpha$_1$-receptors, in rat liver plasma membranes, or at least that alpha$_1$-sites are much more numerous than alpha$_2$-sites. The relative potencies of both prazosin and yohimbine in inhibiting the glycogen phosphorylase stimulated by (−)epinephrine in isolated rat hepatocytes are also shown in Fig. 1. The same order of potencies was found for the inhibition of the enzyme, as for the competition experiments, prazosin (K_i = 0.85 nM) being 13,000-fold more efficient than yohimbine (K_i = 11 μM) (Table 1). Moreover, phenoxybenzamine, reported as more specific for alpha$_1$-sites [9], was very effective in competing with tritiated DHEC ($K_D$app. = 13 nM)* and acted as one of the best antagonists of those tested, for the glycogen phosphorylase activity ($K_i$app. = 0.3 μM).*

From several reports [9, 21–23], it was clear that the two alpha-adrenergic agonists, phenylephrine and methoxamine, were more active at alpha$_1$-receptors than clonidine, other imidazoline derivatives and normetanephrine, which were more active at alpha$_2$ sites. We therefore compared the potencies of these agonists either in competing with DHEC for alpha-sites in rat liver plasma membranes or in stimulating the glycogen phosphorylase activity in isolated rat hepatocytes. When those compounds were tested as competitors against tritiated DHEC, clonidine (K_D = 0.3 μM) and the imidazoline derivatives appeared as the most potent competitors, followed by (−)phenylephrine (K_D = 2 μM), whereas methoxamine (K_D = 10 μM) and normetanephrine (K_D = 66 μM) were almost ineffective in displacing DHEC (Table 1). The same agents were studied for their ability to stimulate the glycogen phosphorylase activity in isolated hepatocytes (Table 1). Some of the activation curves are depicted in Fig. 2. Phenylephrine acted as a full and efficient agonist (K_D = 1 μM), while clonidine, methoxamine and normetanephrine acted as partial agonists with a poor and similar affinity ($K_a \sim 200$ μM). Moreover, clonidine and the related imidazoline derivatives preferentially acted as antagonists upon the (−)epinephrine-stimulated glycogen phosphorylase activity (Table 1), a result already reported in rat parotid cells [24], in human platelets [25, 10] and in rat brain cortical slices [21].

Thus, according to our results, the tritiated DHEC defined alpha-adrenoreceptor of rat liver, which was correlated with the stimulation of glycogen phosphorylase activity, exhibited the criteria necessary to be classified as an alpha$_1$-subtype. A study by Wood *et al.* [11] also supports the concept that the tritiated DHEC binding site of rat liver possesses the characteristics of an alpha$_1$-adrenergic binding site. An anatomic classification into pre- and postsynaptic subtypes did not seem appropriate in the case of rat liver since, for the study of glycogen phosphorylase activity, we used a preparation of isolated hepatocytes devoid of synapses. Thus, we used a pharmacologic classification, based upon the relative potencies of the antagonists, prazosin and yohimbine, and the agonists, phenylephrine and clonidine. Prazosin and phenylephrine are most potent at alpha$_1$-sites, yohimbine and clonidine at alpha$_2$-sites. We wish to point out that clonidine, considered as an agonist for a long time [21], might act differently at alpha$_1$ and alpha$_2$-sites, being a full [21] or a partial [21, 10] agonist at alpha$_2$-sites and an antagonist [24] or a mixed antagonist–agonist at alpha$_1$-sites. Our results indeed illustrate the action of clonidine as a mixed antagonist–agonist at alpha$_1$-sites.

In conclusion, on the basis of the present study, it is clear

* Phenoxybenzamine is an irreversible alpha-blocker. So, the values of K_D and K_i calculated in order to compare that drug with the other drugs tested are only apparent.

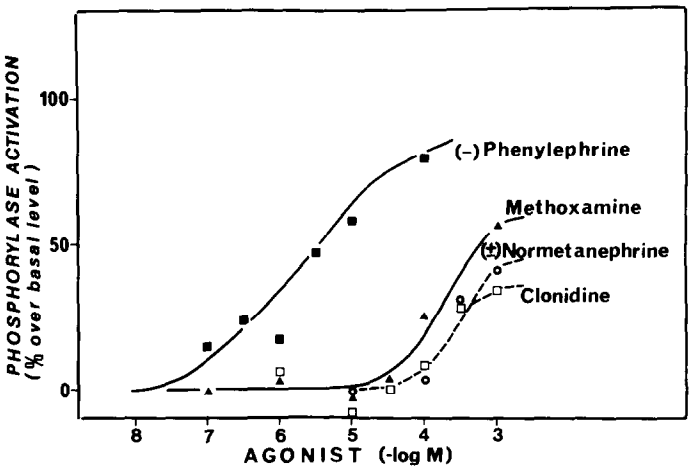

Fig. 2. Activation of the glycogen phosphorylase in rat hepatocytes by alpha-adrenergic agonists. Isolated hepatocytes (52×10^6 cells in 5.8 ml) were preincubated with 4 g/l glucose for 30 min at 37°. Aliquots (125 μl) of cells were incubated with increasing concentrations of (−)phenylephrine (■), methoxamine (▲), (±)normetanephrine (○) or clonidine (□), or with buffer. The incubation at 37° was stopped 2 min later as described in the legend of Fig. 1. Phosphorylase a was assayed as described earlier. Basal level and maximal activity of the enzyme were respectively 0.14 and 0.26 μmole of glucose-1-phosphate transformed in 20 min per mg of protein. The enzyme activity is expressed as per cent of the activity over basal level. Each point is the mean of a quadruplicate determination which agreed within 5 per cent.

that the rat liver alpha-adrenergic receptor, defined by tritiated DHEC binding and by the activation of glycogen phosphorylase, belongs to the alpha$_1$ subtype. It would be of interest to confirm this classification by binding of the agonist epinephrine to the plasma membranes, as proposed by El-Refai *et al.* [26].

Acknowledgements—This work was supported by grants from the Délégation Générale à la Recherche Scientifique et Technique and from the Institut National de la Santé et de la Recherche Médicale. M.A. is the recipient of a fellowship from the Fondation pour la Recherche Médicale Française. We wish to thank Dr. L. P. Aggerbeck for helpful comments and Catherine Petit for her expert secretarial assistance.

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

MARTINE AGGERBECK
GEORGES GUELLAEN
JACQUES HANOUNE

REFERENCES

1. G. Guellaen, M. Yates-Aggerbeck, G. Vauquelin, D. Strosberg and J. Hanoune, *J. biol. Chem.* **253**, 1114 (1978).
2. W. R. Clarke, L. R. Jones and R. J. Lefkowitz, *J. biol. Chem.* **253**, 5975 (1978).
3. N. J. Huston, F. T. Brumley, F. D. Assimacopoulos, S. C. Harper and J. H. Exton, *J. biol. Chem.* **251**, 5200 (1976).
4. M. J. Birnbaum and J. N. Fain, *J. biol. Chem.* **252**, 528 (1977).
5. S. Keppens, J. R. Vandenheede and H. De Wulf, *Biochim. biophys. Acta* **496**, 448 (1977).
6. K. Starke, T. Endo and H. D. Taube, *Naunyn-Schmiedeberg's Arch. Pharmac.* **291**, 55 (1975).
7. J. C. Doxey, C. F. C. Smith and J. M. Walker, *Br. J. Pharmac.* **60**, 91 (1977).
8. S. Z. Langer, *Biochem. Pharmac.* **23**, 1793 (1974).
9. S. Berthelsen and W. A. Pettinger, *Life Sci.* **21**, 595 (1977).
10. J. A. Grant and M. C. Scrutton, *Nature, Lond.* **277**, 659 (1979).
11. C. L. Wood, C. D. Arnett, W. R. Clarke, B. S. Tsai and R. J. Lefkowitz, *Biochem. Pharmac.* **28**, 1277 (1979).
12. D. M. Neville, *Biochim. biophys. Acta* **154**, 540 (1968).
13. P. O. Seglen, in *Methods in Cell Biology* (Ed. D. M. Prescott), pp. 29–83. Academic Press, New York (1976).
14. A. Le Cam, A. Guillouzo and P. Freychet, *Expl. Cell. Res.* **98**, 382 (1976).
15. M. Aggerbeck, G. Guellaen and J. Hanoune, *Br. J. Pharmac.* **62**, 543 (1978).
16. L. Hue, F. Bontemps and H. G. Hers, *Biochem. J.* **152**, 105 (1975).
17. D. P. Gilboe, K. L. Larson and F. Q. Nuttall, *Analyt. Biochem.* **47**, 20 (1972).
18. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
19. Y. Cheng and W. H. Prusoff, *Biochem. Pharmac.* **22**, 3099 (1973).
20. B. B. Hoffman, A. Delean, C. L. Wood, D. D. Schocken and R. J. Lefkowitz, *Life Sci.* **24**, 1739 (1979).
21. H. Schmitt, in *Handbook of Experimental Pharmacology* (Eds. G. V. R. Born, O. Eichler, A. Farah, H. Herken and A. D. Welch), 299–396. Springer, Berlin (1977).
22. J. E. S. Wikberg, *Nature, Lond.* **273**, 164 (1978).
23. G. M. Drew, *Eur. J. Pharmac.* **42**, 123 (1977).
24. J. N. Davis and W. Maury, *J. Pharmac. exp. Ther.* **207**, 425 (1978).
25. K. H. Jakobs, *Nature, Lond.* **274**, 819 (1978).
26. M. F. El-Refai, P. F. Blackmore and J. H. Exton, *J. biol. Chem.* **254**, 4375 (1979).