Biochemical Pharmacology, Vol. 32, No. 1, pp. 69–71, 1983.
Printed in Great Britain.

0006-2952/83/010069-03 $03.00/0

GUANINE NUCLEOTIDES AND ALPHA$_1$ ADRENERGIC RECEPTORS IN THE HEART

GARY L. STILES,* BRIAN B. HOFFMAN,† MARGARET HUBBARD, MARC G. CARON and ROBERT J. LEFKOWITZ

Howard Hughes Medical Institute, Departments of Medicine (Division of Cardiology) and Biochemistry, Duke University Medical Center, Durham, NC 27710, U.S.A.

(*Received* 13 *February* 1982; *accepted* 19 *May* 1982)

Abstract—Guanine nucleotides such as GTP and Gpp(NH)p are known to regulate the affinity of beta and alpha$_2$ adrenergic receptors for agonists as assessed by radioligand binding techniques. Recent studies in the rat heart using the radioligand [^{3}H]WB4101, which reportedly labels alpha$_1$ adrenergic receptors, have suggested that the affinity of alpha$_1$ adrenergic receptors for epinephrine is altered by guanine nucleotides. To assess the possibile role of guanine nucleotides in alpha$_1$ adrenergic function, we have constructed (−)epinephrine competition curves in the absence and presence of guanine nucleotides using the highly alpha$_1$ subtype selective agents [^{3}H]prazosin and [^{125}I]BE2254 in rat cardiac membranes. Epinephrine competition curves in the absence of guanine nucleotides were steep and uniphasic in character, and the addition of Gpp(NH)p (10^{-4} M) had no effect on the ability of epinephrine to compete for either [^{3}H]prazosin or [^{125}I]BE2254 binding sites. In this same membrane preparation, the ability of (−)isoproterenol to compete with [^{3}H]dihydroalprenolol for beta adrenergic receptors binding sites was decreased significantly by Gpp(NH)p. These findings demonstrate that, under conditions where guanine nucleotide regulation of agonist–receptor binding in these membranes can be observed, no nucleotide regulation of agonist–alpha$_1$ receptor interactions was evidenced using subtype selective radioligands. These results suggest that previous reports of agonist–alpha$_1$ receptor regulation by guanine nucleotides may represent a manifestation of the anomalous binding characteristics of [^{3}H]WB4101 as compared with [^{3}H]prazosin and [^{125}I]BE2254.

Receptor-mediated regulation of adenylate cyclase activity has been demonstrated to be a guanine nucleotide-dependent process [1]. This has been shown for both stimulation of adenylate cyclase by beta$_1$ and beta$_2$ adrenergic receptors and inhibition of adenylate cyclase by alpha$_2$ adrenergic receptors [1]. In addition, the binding affinity of agonists such as isoproterenol or epinephrine for these receptors is selectively decreased by guanine nucleotides [2–4]. By contrast, the alpha$_1$ adrenergic receptor appears not to be coupled to adenylate cyclase but, rather, may alter cellular calcium fluxes to produce a physiologic effect [5]. There is no evidence that this process is modulated by guanine nucleotides.

Recently, however, it has been suggested that the affinity of alpha$_1$ adrenergic receptors for epinephrine in rat heart [6, 7] and vascular tissue [8] is decreased by guanine nucleotides, as assessed by agonist competition curves using the alpha receptor antagonist ligand [^{3}H]WB4101. These findings appear to represent the first description of a guanine nucleotide sensitive alpha$_1$ adrenergic receptor binding process. The radioligand [^{3}H]WB4101, however, has been shown not to be subtype selective in some tissues such as the rabbit uterus [9] while maintaining greater selectivity in others such as calf cerebral cortex [10]. To critically assess whether guanine nucleotides truly modulate agonist binding at alpha$_1$ adrenergic receptors in rat myocardial membranes, we utilized the highly alpha$_1$ receptor subtype selective agents [^{3}H]prazosin [11, 12] and [^{125}I]BE2254 [13–15].

METHODS

Male Sprague–Dawley rats (200–300 g) were obtained from the Charles River Breeding Laboratories (Wilmington, MA). Animals were decapitated, and the hearts were removed and washed in ice-cold saline (0.9%). Myocardial membranes were prepared as previously described [16]. [^{3}H]Prazosin (33 Ci/mmole) was obtained from the Amersham Corp. (Arlington Heights, IL). [^{125}I]BE2254 was prepared according to the method of Engel and Hoyer [15] to a specific activity of 2200 Ci/mmole. [^{3}H]-Dihydroalprenolol was obtained from the New England Nuclear Corp. (Boston, MA) with a specific activity of 47 Ci/mmole.

Assays for [^{3}H]prazosin were performed in a total volume of 0.9 ml containing 0.6 ml of membranes in 50 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 0.15 ml of labeled ligand and 0.15 ml of H_2O or competing ligand. Incubations were for 30 min at 25° followed by vacuum filtration and a 20-ml wash with cold buffer.

Assay for [^{3}H]DHA was performed as previously described [16]. [^{125}I]BE2254 binding assays were per-

* Send reprint requests to: Dr. Gary L. Stiles, P.O. Box 3444, Duke University Medical Center, Durham, NC 27710, U.S.A.

† Current address: Division of Clinical Pharmacology (S-155), Stanford University Medical Center, Stanford, CA 94305, U.S.A.

formed in a 0.5 ml assay with 150 μl of membranes in 50 mM Tris–HCl (pH 7.5), 10 mM MgCl and incubated for 60 min at 25° followed by vacuum filtration as described above.

Computer modeling of competition curves was performed using a non-linear least squares curve fitting technique to determine K_D values and a four parameter logistic equation to determine EC_{50} values and slope factors as previously described [17].

RESULTS

It has been demonstrated previously that agonist–beta-adrenergic receptor interactions are regulated by guanine nucleotides in rat myocardial membranes [18]. In the studies to be reported, we first documented in the membrane preparation used that the guanine nucleotide sensitivity of agonist–beta-adrenergic receptor interactions was intact. In Fig. 1, the competition curves of isoproterenol with the beta-adrenergic receptor antagonist ligand [^{3}H]DHA are shown in the presence and absence of Gpp(NH)p (10^{-4} M). In the absence of guanine nucleotides, the curve is shallow with a slope factor of 0.45 ± 0.11 (mean ± S.E.M.). The EC_{50} for this curve is 0.23 ± 0.13 μM. In the presence of Gpp(NH)p, the curve steepens to a slope factor of 0.87 ± 0.2 (not significantly different than 1) and shifts to the right with an EC_{50} of 1.5 ± 0.4 μM. This represents a significant ($P < 0.013$) 6.5-fold shift to the right. These changes demonstrate that the regulation of agonist–beta-adrenergic receptor interactions was intact in this membrane preparation.

Competition curves of (−)epinephrine with the alpha$_1$ subtype selective antagonist [^{3}H]prazosin in the presence and absence of Gpp(NH)p were constructed in this same membrane preparation. The subtype selectivity of [^{3}H]prazosin for alpha$_1$ receptors in rat myocardial membranes has been described previously [11]. The K_D for antagonist prazosin is 87 ± 5 pM in both the absence and presence of

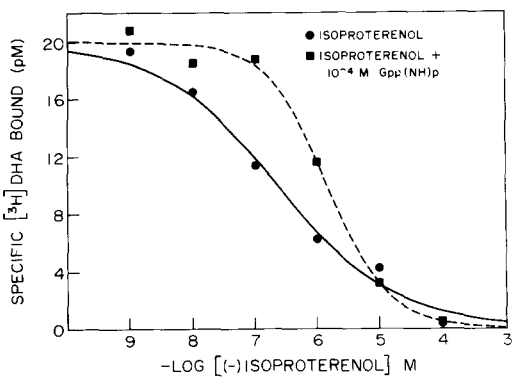

Fig. 1. Competition curves of (−)isoproterenol with [^{3}H]dihydroalprenolol in the presence and absence of guanine nucleotides. Myocardial membranes were incubated with 2.0 nM [^{3}H]dihydroalprenolol in competititon with the indicated concentrations of isoproterenol in the presence and absence of Gpp(NH)p (10^{-4} M). The data represent the mean of three separate experiments with each point determined in duplicate. The percent specific binding in these experiments was 60–65% of the total binding as determined by counts competed for by 10^{-3} M isoproterenol. Propranolol at 10^{-5} M gave similar results.

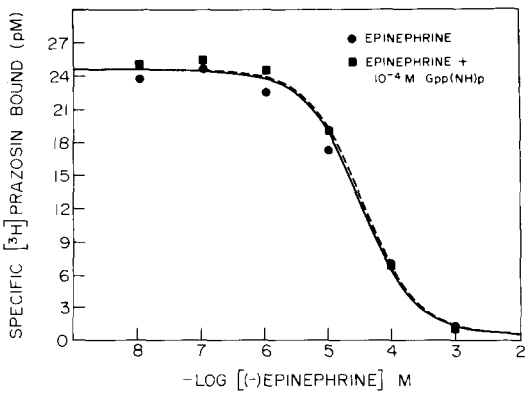

Fig. 2. Competition curves of (−)epinephrine with [^{3}H]prazosin in the presence and absence of guanine nucleotides. Myocardial membranes were incubated with 0.3 to 0.4 nM [^{3}H]prazosin and competing ligand, as indicated, with and without Gpp(NH)p (10^{-4} M). This experiment is representative of four separate experiments with each point determined in duplicate. The percent specific binding in these experiments was 80–85% of the total binding as determined with 10^{-5} M phentolamine or 10^{-3} M epinephrine, both of which gave similar results.

Gpp(NH)p. As can be seen in Fig. 2, the curves in the presence and absence of guanine nucleotides are virtually identical. Both curves are steep with a slope factor of 0.85 ± 0.10 (not significantly different than 1). The EC_{50} values or the K_D values calculated for the competing drug, epinephrine, were not significantly different in the presence and absence of Gpp(NH)p (K_D values = 2.3 ± 0.5 vs 2.6 ± 0.5 μM respectively). It is apparent from these data that the agonist–alpha$_1$ receptor interactions as assessed by [^{3}H]prazosin binding were not regulated by guanine nucleotides under conditions where agonist–beta-adrenergic receptors were modulated by guanine nucleotides.

To further validate this finding, similar experiments were conducted using the new alpha$_1$ subtype selective antagonist [^{125}I]BE2254. This ligand has been characterized and shown to have all the properties of an alpha$_1$ subtype selective radioligand [13–15]. In this system, the K_D value for [^{125}I]BE2254 is 73 ± 14 pM in the absence and presence of Gpp(NH)p. Figure 3 illustrates the epinephrine competition curves in the presence and absence of Gpp(NH)p. Again, it is apparent that the agonist–receptor interactions were not modulated by guanine nucleotides. The EC_{50} or the calculated K_D values for epinephrine were not different in the absence (K_D = 7.1 ± 1.7 μM) or in the presence of Gpp(NH)p (K_D = 9.4 ± 2.3 μM). The slope factors in the absence (0.99 ± 0.20) and in the presence (0.86 ± 0.22) of Gpp(NH)p are not significantly different nor are they significantly different than 1.

These two independent sets of data suggest that guanine nucleotides do not regulate agonist–alpha$_1$ receptor interactions in rat myocardium.

DISCUSSION

Guanine nucleotides are known to be important in the function of receptor-mediated regulation of

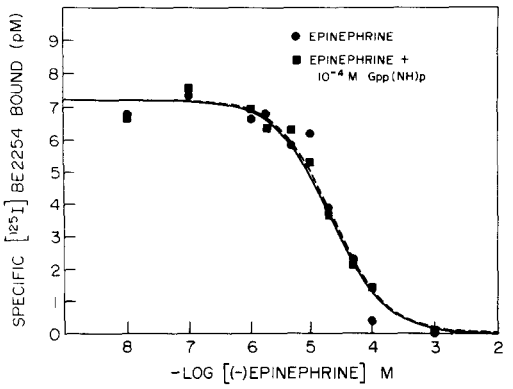

Fig. 3. Competition of (−)epinephrine with [^{125}I]BE2254 in the presence and absence of guanine nucleotides. Myocardial membranes were incubated with 0.1 nM [^{125}I]BE2254 and competing ligand, as indicated, with or without Gpp(NH)p (10^{-4} M). This experiment is representative of two separate experiments with each point determined in duplicate. The percent specific binding in these two experiments was 60 and 69% as determined with 10^{-3} M epinephrine or 10^{-5} M phentolamine, both of which gave similar results.

adenylate cyclase [1]. Two demonstrable effects are their absolute requirement for effective activation or inhibition of adenylate cyclase by beta adrenergic receptors or alpha$_2$ adrenergic receptors, respectively, and the ability of guanine nucleotides to decrease the apparent affinity of agonists for their receptors [2, 3]. For the alpha$_2$ adrenergic receptor, this has been demonstrated in several tissues including the human platelet [4], the rabbit uterus [4] and the rat liver [12]. In contrast, alpha$_1$ adrenergic receptors which are not coupled to adenylate cyclase have been shown not to be regulated by guanine nucleotides in the rat liver [12] and the rabbit uterus [4]. These studies utilized the subtype selective agent [^{3}H]prazosin as well as the non-subtype selective ligand [^{3}H]dihydroergocryptine.

In this study, we have demonstrated that in rat myocardial membranes agonist–alpha$_1$ adrenergic receptor interactions are not regulated by guanine nucleotides. This was shown with two separate alpha$_1$ subtype selective agents, [^{3}H]prazosin and [^{125}I]BE2254. In addition, we have demonstrated that this was not simply an artifact of the membrane preparation used since agonist–beta adrenergic receptor interactions were clearly regulated by guanine nucleotides under the same conditions.

These findings are in contrast to previously published studies of alpha$_1$ adrenergic receptors in rat heart [6, 7] and vascular tissues [8] using the radioligand [^{3}H]WB4101 in which agonist–alpha adrenergic receptor interactions appeared to be slightly regulated by guanine nucleotides. One possible reason for these disparate results is most likely related to the use of [^{3}H]WB4101 as the radioligand. [^{3}H]WB4101 has been demonstrated not to have subtype selectivity in some tissues [9] where it binds with equal affinity to alpha$_1$ and alpha$_2$ receptors. In other systems [19], it also appears to bind to low affinity non-alpha adrenergic receptor sites. This suggests that, for each system where [^{3}H]WB4101 is utilized, the alpha receptor binding must be carefully characterized. Studies in the rat heart have shown that antagonist competition curves of either prazosin or phentolamine with [^{3}H]WB4101 are shallow, suggesting that [^{3}H]WB4101 may label several populations of binding sites, only one of which has the characteristics of alpha receptors [20]. In addition, previous reports from this laboratory have documented that there is no evidence for alpha$_2$ adrenergic receptors in rat myocardium [11]. This heterogeneity of binding with [^{3}H]WB4101 makes interpretation of the effects of guanine nucleotides on alpha$_1$ receptors difficult since one cannot be sure which receptor binding site(s) the guanine nucleotides are acting upon.

In conclusion, the present data suggest that agonist–alpha$_1$ receptor interactions in rat myocardium are not regulated by guanine nucleotides and are consistent with the notion that the radioligands [^{3}H]prazosin and [^{125}I]BE2254 have subtype selective properties superior to that of [^{3}H]WB4101.

Acknowledgements—We wish to thank Ms. Donna Addison for her careful preparation of the manuscript. Dr. Stiles is supported in part by N.I.H. Grant HL 07101. Dr. Lefkowitz is an Investigator of the Howard Hughes Medical Institute. This work was also supported in part by grants from the N.I.H. (HL 16037 and HL 20339).

REFERENCES

1. M. Rodbell, *Nature, Lond.* **284**, 17 (1980).
2. M. E. Maguire, P. M. Van Arsdale and A. G. Gilman, *Molec. Pharmac.* **12**, 335 (1976).
3. R. J. Lefkowitz, D. Mullikin and M. G. Caron, *J. biol. Chem.* **251**, 4686 (1976).
4. B. B. Hoffman, D. Mullikin Kilpatrick and R. J. Lefkowitz, *J. biol. Chem.* **255**, 4645 (1980).
5. J. N. Fain and J. A. Garcia-Sainz, *Life Sci.* **26**, 1183 (1980).
6. S. Yamada, H. I. Yamamura and W. R. Roeske, *Eur. J. Pharmac.* **63**, 239 (1980).
7. K. Yamanaka, H. I. Yamamura and W. R. Roeske, *Circulation* **64**, IV-206 (1981).
8. W. S. Colucci, M. Kay, M. A. Gimbrone, Jr. and R. W. Alexander, *Circulation* **64**, IV-206 (1981).
9. B. B. Hoffman and R. J. Lefkowitz, *Biochem. Pharmac.* **29**, 1537 (1980).
10. D. C. U'Prichard and S. H. Snyder, *J. biol. Chem.* **253**, 3444 (1978).
11. R. S. Williams, D. F. Dukes and R. J. Lefkowitz, *J. cardiovasc. Pharmac.* **3**, 522 (1981).
12. B. B. Hoffman, D. F. Dukes and R. J. Lefkowitz, *Life Sci.* **28**, 265 (1981).
13. H. Glossman, F. Lubbecks and P. Belleman, *Naunyn-Schmiedeberg's Archs Pharmac.* **318**, 1 (1981).
14. M. Gothart, J. Nolte and G. Weinheimer, *Eur. J. Pharmac.* **70**, 35 (1981).
15. G. Engel and D. Hoyer, *Eur. J. Pharmac.* **73**, 221 (1981).
16. L. T. Williams, R. J. Lefkowitz, A. M. Watanabe, D. R. Hathaway and H. R. Besch, *J. biol. Chem.* **252**, 2787 (1977).
17. R. S. Kent, A. De Lean and R. J. Lefkowitz, *Molec. Pharmac.* **17**, 14 (1980).
18. G. L. Stiles and R. J. Lefkowitz, *Life Sci.* **28**, 2529 (1981).
19. T. F. Lyon and W. C. Randall, *Life Sci.* **26**, 1121 (1980).
20. S. Yamada, H. I. Yamamura and W. R. Roeske, *J. Pharmac. exp. Ther.* **45**, 176 (1980).