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SHORT COMMUNICATIONS

# An Agonist-Specific Effect of Guanine Nucleotides on Binding to the *Beta* Adrenergic Receptor

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SUMMARY

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We have previously characterized the binding of [$^{125}$I]iodohydroxybenzylpindolol to *beta* adrenergic receptor sites in two clonal lines of cultured cells [Maguire, M. E., Wiklund, R. A., Anderson, H. J. & Gilman, A. G. (1976) *J. Biol. Chem.*, 251, in press]. We now report that whereas guanine nucleotides have no effect on the binding of this iodinated antagonist, GTP, GDP, and guanylyl imidodiphosphate all impair the ability of *beta* adrenergic *agonists* to inhibit the binding of [$^{125}$I]iodohydroxybenzylpindolol. The nucleotides have no effect on the ability of other *beta* adrenergic *antagonists* to inhibit binding. Thus, with this method, *beta* adrenergic agonists and antagonists can be distinguished by means of binding alone; no assessment of "effect" need be made.

## INTRODUCTION

Guanine nucleotides are known to modulate the activity of adenylate cyclase (EC 4.6.1.1) *in vitro*. In a variety of systems they have been shown to stimulate or inhibit both basal and hormone-activated enzyme activity (1-4). Rodbell and colleagues have previously reported that GTP and other guanine nucleotides decrease the affinity of hepatic glucagon receptors for [$^{125}$I]glucagon (1) and, at the same time, increase the apparent affinity of glucagon for adenylate cyclase (5). Aurbach and colleagues (6, 7) have reported data on the effect of guanine nucleotides on the *beta* adrenergic receptor-adenylate cyclase system of turkey erythrocytes; these authors utilized [$^{125}$I]IHYP,[1] a *beta* adrenergic antagonist, as the ligand to quantitate the receptor. In this system the apparent affinities of *beta* adrenergic antagonists for the adenylate cyclase and their binding constants for the receptor were in close agreement; however, in the absence of a guanine nucleotide the dissociation constants for binding of agonists were 10-fold lower than their activation constants for adenylate cyclase. The addition of nucleotide resulted in an improvement of the apparent affinity for the cyclase to the same value as that obtained by

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[1] The abbreviations used are: [$^{125}$I]IHYP, [$^{125}$I]iodohydroxybenzylpindolol; HYP, hydroxybenzylpindolol (*dl*-3-indoloxy-1-[2-*p*-hydroxybenzylpropyl-2-amino]isopropanol); GMP-PNP, guanylyl imidodiphosphate.

measurements of binding to the receptor. The latter affinities did not change in the presence of nucleotide.

We have also previously studied the binding of [$^{125}$I]IHYP to *beta* adrenergic receptor sites in two cultured cell clones, C6TG1A, a rat glioma, and VA2, an $SV_{40}$-transformed human lung fibroblast (8). Appropriate receptor sites in these cell lines were identified by several criteria, including stereoselective inhibition of binding by *beta* adrenergic antagonists and agonists, correlation between the potency of these agents to affect adenylate cyclase activity and to inhibit binding of [$^{125}$I]IHYP, correlation of the kinetics of binding of [$^{125}$I]IHYP with the kinetics of adenylate cyclase activation, and correlation of binding activity with the presence or absence of response to catecholamines (i.e., stimulation of adenylate cyclase) in various cell clones.

Guanine nucleotides have no significant effect on the binding of [$^{125}$I]IHYP in these cells. We now report, however, that these nucleotides specifically decrease the affinity of *beta* adrenergic agonists for the receptor while having no effect on the affinities of antagonists.

The methodology has been reported in detail (8). Briefly, HYP was iodinated with carrier-free Na$^{125}$I and [$^{125}$I]IHYP was separated chromatographically from HYP, resulting in ligand presumably purified to the theoretical specific activity of 2200 Ci/mmole. Crude particulate preparations containing a high density of *beta* adrenergic receptors were prepared by homogenization and sedimentation between 200 and 30,000 × *g*. Binding was assayed by incubation of this particulate preparation at 30° in 50 mM sodium *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonate (pH 8.0) and 4 mM $MgSO_4$ in a final volume of 500 μl, usually containing 25 pM [$^{125}$I]IHYP (45,000 cpm). Reactions were initiated by addition of protein. After 30 or 60 min, as noted, the mixture was diluted with 1 ml of 37° wash buffer (20 mM potassium phosphate, pH 8.0, and 1 mM $MgSO_4$) containing 100 μM *dl*-propranolol, and filtered through a glass fiber filter (Gelman, type A/E) under low vacuum. The filter was rinsed with 25 ml of wash buffer at 37° and counted in a Beckman γ-counter. Specific binding was defined as the difference in radioactive ligand bound in the presence (during the incubation) and absence of 1 μM *l*-propranolol. (Propranolol, 100 μM, was added to all samples immediately prior to filtration to minimize the binding of ligand to the filter; this procedure did not alter binding of [$^{125}$I]IHYP to the protein during the brief exposure.) Results are reported as the percentage of total specific binding observed; specific binding usually represented a total difference of 2500 cpm at a [$^{125}$I]IHYP concentration of 25 pM and a protein concentration of 150 μg/ml, while nonspecific binding was approximately 400 cpm. Replicate samples agreed within 5%.

Figures 1 and 2 show the effect of GMP-PNP on [$^{125}$I]IHYP binding in the presence and absence of *l*-epinephrine and *l*-alprenolol in C6TG1A (Fig. 1) and of *l*-epinephrine and *l*-propranolol in VA2 (Fig. 2). GMP-PNP decreased the affinity of *l*-epinephrine in both cell lines by nearly 10-fold but did not change the affinity of *l*-propranolol or *l*-alprenolol. In seven exper-

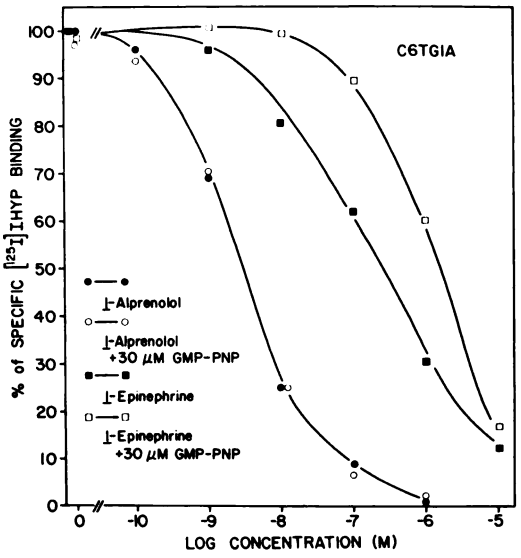


FIG. 1. *Effect of 30 μM GMP-PNP on competition by l-epinephrine and l-alprenolol for [$^{125}$I]IHYP binding sites in C6TG1A particulate fraction*

Incubation at 30° was conducted for 60 min. See the text and ref. 8 for details of the binding assay.

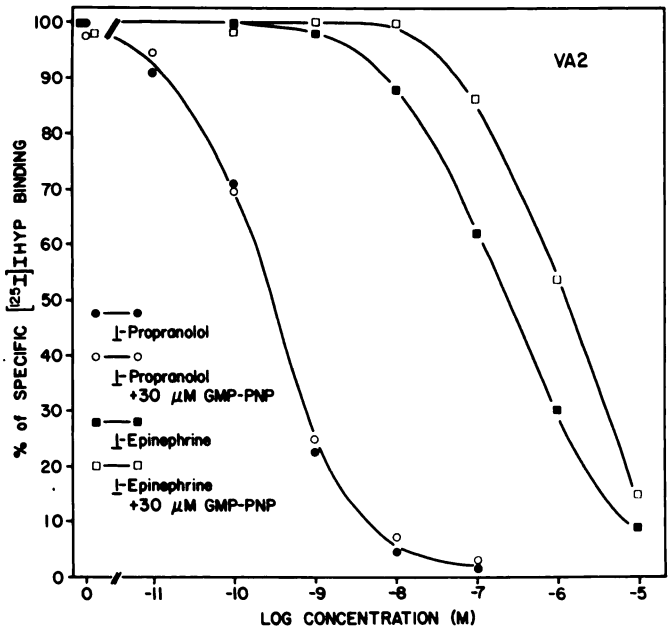


FIG. 2. *Effect of 30 μM GMP-PNP on competition by l-epinephrine and l-propranolol for [125I]IHYP binding sites in VA2 particulate fraction*

Incubation at 30° was carried out for 60 min.

iments with C6TG1A, 30 μM GMP-PNP shifted the apparent $K_D$ for isoproterenol from 30 ± 13 to 260 ± 160 nM ($p < 0.002$). The shift was observed in all experiments and was never less than 5-fold.

This apparent specificity for an agonist was investigated with several other compounds (Table 1). In C6TG1A cells GMP-PNP decreased the affinity of *l*-isoproterenol, *l*-epinephrine, *l*-norepinephrine, *l*-salbutamol, *l*-phenylephrine, metaproterenol, and terbutaline, all adrenergic agonists. GMP-PNP had no detectable effect on the affinities for binding of the antagonists *l*-alprenolol, *l*-sotalol, *d*-sotalol, *l*-tolamolol, *dl*-pindolol, *dl*-HYP, or *l*-1-(4'-nitrophenyl)-2-isopropylaminoethanol. There is no apparent relationship between the presence or magnitude of the shift in affinity and the potency of the compound. Interestingly, the binding of quinterenol, which has been reported to be a *beta*$_2$ agonist in humans, was not shifted by GMP-PNP in the C6TG1A fraction; the compound did compete with [125I]IHYP for receptor sites. Subsequently we found that quinterenol, in intact C6TG1A cells, is a *beta* adrenergic antagonist. Agonistic activity was not noted to a concentration of 0.1 mM (data not shown), thus confirming the lack of effect of GMP-PNP.

It is to be noted that in both C6TG1A and VA2 cells the addition of nucleotide had no effect on the quantity of [125I]IHYP bound in the absence of competing agonists or antagonists. The concentration of [125I]IHYP used in these experiments (25 pM) was much less than the apparent $K_D$ in C6TG1A (200 pM) and slightly higher than the $K_D$ in VA2 (15 pM).

GTP, GDP, and ITP also caused the same specific shift in affinity (not shown). The magnitude of this shift was related to the concentration of nucleotide, since increasing concentrations of GTP (not shown) or GMP-PNP (Fig. 3) progressively decreased the apparent binding constant for *l*-isoproterenol. The effect was maximal at about 30 μM GTP or GMP-PNP. Experiments done in the absence of $Mg^{2+}$ (with 0.1 mM EDTA added) suggest that the effect of GMP-PNP is not dependent on the presence of $Mg^{2+}$. Preliminary data also suggest that this divalent cation changes

TABLE 1

*Effect of GMP-PNP on affinities of various agonists and antagonists for beta receptor of C6TG1A cells*

For isoproterenol, the $K_D$ values are the means ± standard errors of seven experiments and are significantly different ($p < 0.002$ by $t$-test). For all other drugs, data from a single experiment are shown. For *beta* adrenergic antagonists, there was no detectable change in $K_D$ in the presence of 30 μM GMP-PNP. For agonists, excluding isoproterenol, the ratio of the $K_D$ with GMP-PNP to the $K_D$ in the absence of GMP-PNP was calculated and found to be significantly different from unity ($p < 0.001$ by $t$-test).

| Compound | $K_D$ | |
|---|---|---|
| | Control | +30 μM GMP-PNP |
| | *nM* | *nM* |
| Agonists | | |
| *l*-Isoproterenol | 30 ± 13 | 260 ± 160 |
| *l*-Epinephrine | 200 | 2,000 |
| *l*-Norepinephrine | 300 | 3,000 |
| *l*-Salbutamol | 1,000 | 4,000 |
| *l*-Phenylephrine | 5,000 | 30,000 |
| *dl*-Metaproterenol | 3,000 | 10,000 |
| *dl*-Terbutaline | 3,000 | 20,000 |
| Antagonists | | |
| *l*-Propranolol | 2 | 2 |
| *l*-Alprenolol | 5 | 5 |
| *dl*-HYP | 0.2 | 0.2 |
| *dl*-Pindolol | 2 | 2 |
| *l*-1-(4'-Nitrophenyl)-2-isopropylamino-ethanol | 700 | 700 |
| *l*-Sotalol | 300 | 300 |
| *d*-Sotalol | 30,000 | 30,000 |
| *l*-Tolamolol | 30 | 30 |
| Quinterenol | 10,000 | 10,000 |

the apparent affinity of *l*-isoproterenol for the receptor.

The effects of guanine nucleotides on the adenylate cyclases of C6TG1A and VA2 are complex. The simplest prediction is that the shift to the right for inhibition of binding of [$^{125}$I]IHYP by isoproterenol would be reflected in a corresponding shift in isoproterenol-stimulated adenylate cyclase activity. Preliminary data suggest that the nucleotide effect is not this simple. Basal enzyme activity was inhibited at low concentrations of GMP-PNP but was increased more than 2-fold at 0.1 mM. GTP only stimulated basal activity, and ITP had no effect on basal rates of synthesis. Concentration curves for isoproterenol in the presence of ITP were shifted slightly to the right, as was binding, but the maximal stimulation caused by isoproterenol was decreased (data not shown). Thus, while the correlation between effects of nucleotides on binding as opposed to enzyme activity may be difficult to demonstrate with clarity, we feel that the observations on binding alone are significant. We are able to distinguish between agonists and antagonists without a determination of effect; these and further experiments should provide additional insight into the nature of the interaction between receptor and adenylate cyclase.

The agonist-specific effect of guanine nucleotides noted here may be interpreted in terms of current models of adenylate cyclase (9, 10). We provide an example of such interpretation without any attempt to evaluate multiple possibilities at this preliminary stage. Guanine nucleotides are presumed to occupy a site on some component of the adenylate cyclase system other than the receptor. Although this is an assumption, it is suggested by the find-

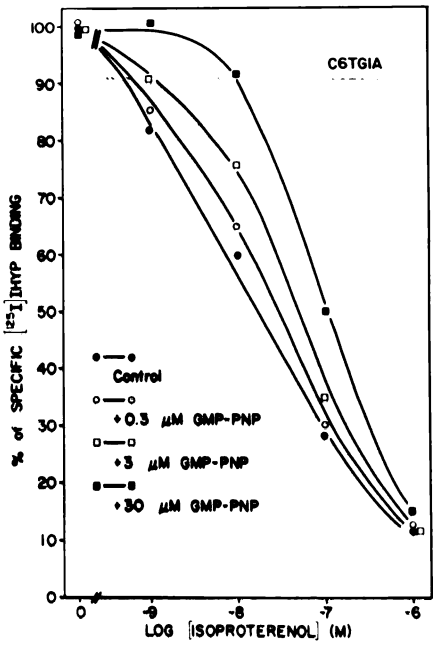


FIG. 3. *Effect of various concentrations of GMP-PNP on competition by l-isoproterenol for [$^{125}$I]IHYP binding sites in C6TG1A particulate fraction*

Incubation at 30° was carried out for 60 min.

ing that these nucleotides can alter basal enzyme activity in a variety of systems, regardless of the type of receptor present (1–5, 11) and including enzymes for which hormonal regulation has not been detected. The nucleotide may be bound directly to the catalytic moiety or to a separate, hypothetical component (9). It is further envisioned that the binding of an agonist to the receptor initiates some form of interaction (coupling) between the receptor and the guanine nucleotide-binding component of the system. (A similar formulation envisions the agonist interacting only with a form of the receptor that is coupled to the nucleotide-binding component.) Because the receptor and the nucleotide-binding moiety are or become coupled, occupation by a guanine nucleotide of the appropriate regulatory site can effect a change in the characteristics of the binding of hormone to receptor.

Suppose, then, at least in C6TG1A and VA2 cells, that a *beta* adrenergic antagonist exerts its effect simply by occupying the receptor site, without initiating the coupling process. Then the binding of any antagonist could *not* be affected by guanine nucleotides, since the receptor and the nucleotide-binding component of the system are not coupled. Thus, by this hypothesis, in C6TG1A and VA2 cells, the ability of GMP-PNP or GTP to affect [$^{125}$I]IHYP binding might be envisioned as a measure of coupling between the receptor and the guanine nucleotide-binding moiety.

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