MOLECULAR PHARMACOLOGY, 22:298–303

Dopamine Receptor of the Porcine Anterior Pituitary Gland

Effects of *N*-Ethylmaleimide and Heat on Ligand Binding Mimic the Effects of Guanine Nucleotides

BRIAN F. KILPATRICK, ANDRE DE LEAN,[1] AND MARC G. CARON

Departments of Medicine (Cardiology) and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

Received March 17, 1982; Accepted May 11, 1982

SUMMARY

The dopaminergic receptor of the porcine anterior pituitary gland exhibits two different affinity states discriminated by both agonists and antagonists and interconvertible by guanine nucleotides. These effects of guanine nucleotides on the interaction of ligands with the receptor can be mimicked by treatments such as *N*-ethylmaleimide and heat under conditions that have been shown to inactivate specifically guanine nucleotide regulatory functions in other receptor systems. Both *N*-ethylmaleimide (<100 μM) and heat treatments (53° for 4 min) of membrane preparations (a) shift to the right and steepen the competition curves of agonists for [^{3}H]spiroperidol binding, (b) decrease the direct binding of the agonist [^{3}H]*n*-propylapomorphine to the agonist high-affinity form of the receptor, and (c) lead to an apparent increase in the binding of the antagonist [^{3}H] spiroperidol at low concentrations of the ligand. These effects are selective for the form of the receptor postulated to bind agonists with high affinity and [^{3}H]spiroperidol with low affinity. The total [^{3}H]spiroperidol number of binding sites is unaffected by those treatments. However, at higher concentrations of *N*-ethylmaleimide (>1 mM) and longer heat exposure (>10 min), binding of the antagonist [^{3}H]spiroperidol is decreased markedly, presumably a reflection of inactivation of the receptor itself. The differential effects of these treatments (*N*-ethylmaleimide and heat) on high-affinity agonist/low-affinity antagonist binding and the over-all antagonist binding, coupled with the similarity in the characteristics of inactivation of nucleotide regulatory functions in other receptor systems, lend indirect but strong support for the involvement of a guanine nucleotide-binding protein in the interaction of the dopamine receptor of the porcine anterior pituitary gland with dopaminergic ligands.

INTRODUCTION

In several receptor systems guanine nucleotides have been shown to modulate agonist binding (1). Dopaminergic receptors of the D_2, or butyrophenone-specific, subtype in the corpus striatum (2–4), bovine anterior pituitary gland (5, 6), and intermediate lobe of bovine pituitary gland (7) have also been shown to be regulated by guanine nucleotides. In the presence of guanine nucleotides, agonist competition curves for [^{3}H]spiroperidol binding shift to the right and steepen, indicating a decrease in the apparent affinity of the receptor for agonists. Quantitative analysis by computer modeling of ligand binding to porcine anterior pituitary dopamine receptors indicates that the receptor exists in two affinity forms for agonists and that guanine nucleotides cause a transition of the agonist high-affinity form to a low-affinity form (8).

By analogy with other receptor systems, these specific nucleotide-mediated effects on receptor binding suggest the involvement of a guanine nucleotide regulatory protein with the dopaminergic receptor entity. A similar guanine nucleotide-mediated transition of agonist affinity forms has been well-documented in the adenylate cyclase-coupled *beta*-adrenergic receptor system (9–11). The biochemical events of this phenomenon have been elucidated, and it appears that binding of an agonist to the receptor induces or stabilizes the interaction of the agonist-receptor complex with a guanine nucleotide regulatory protein resulting in a high-affinity agonist-receptor complex (12, 13). Binding of a guanine nucleotide triphosphate to the regulatory protein destabilizes this ternary complex, thus lowering the affinity of the receptor for the agonist (13). NEM,[2] a sulfydryl alkylating

This work was supported in part by Grant NS-06233 from the National Institutes of Health.

[1] Recipient of a Centennial Postdoctoral Fellowship from the Medical Research Council of Canada. Present address, Clinical Research Institute of Montreal, Montreal Que., Canada H2W 1R7.

[2] The abbreviation used is: NEM, *N*-ethylmaleimide.

0026-895X/82/050298-06$02.00/0

agent, has been found to mimic the effect of nucleotides on agonist binding in the *beta*-adrenergic receptor of frog erythrocytes. In this system, treatment of membranes with NEM prevents the formation of the agonist high-affinity receptor complex (14) without any effect on antagonist binding to the receptor. Previously, Ross and Gilman (15) showed by reconstitution studies of hormone and nucleotide responsiveness of adenylate cyclase in S-49 lymphoma cells that the guanine nucleotide regulatory protein activity was exquisitely thermolabile at 50° with a $t_{1/2}$ of inactivation of 2–8 min. As shown by Stadel *et al.* (16) in the frog erythrocyte system, the nucleotide regulatory protein activity assayed by reconstitution studies is believed to be the same functional entity which is involved in high-affinity agonist binding.

In order to gain some insights into the nature of the dopaminergic agonist high-affinity receptor complex and the possible involvement of a guanine nucleotide regulatory protein, we have characterized and compared the effects of guanine nucleotides, NEM, and heat treatments on agonist and antagonist binding in porcine anterior pituitary membranes.

EXPERIMENTAL PROCEDURES

Materials

[^{3}H]Spiroperidol (33.2–51 Ci/mmole) and [^{3}H]*N-n*-propylapomorphine (~60 Ci/mmole) were purchased from New England Nuclear Corporation (Boston, Mass.). NEM was obtained from Sigma Chemical Company (St. Louis, Mo.). Sources of other materials were as described in the accompanying paper (8).

Methods

Membrane preparation. Fresh female porcine pituitary glands were obtained from a local slaughterhouse and were kept in 2 mM $MgCl_2$/25 mM Tris-HCl (pH 7.4 at 0–4°) until preparation of the membranes (3–5 hr after slaughter). The anterior lobes were separated from the posterior and intermediate lobes, minced, and homogenized with 12 strokes of a glass-Teflon homogenizer in 250 mM sucrose/2 mM $MgCl_2$/25 mM Tris-HCl (pH 7.4 at 4°) (20 ml/g of tissue) (Buffer A). All steps were performed at 0–4°. The homogenate was filtered through two layers of cheesecloth and centrifuged at 300 × *g* for 10 min. The supernatant was retained and the pellet was rehomogenized and centrifuged again. The combined supernatants were layered over 50% sucrose/2 mM $MgCl_2$/50 mM Tris-HCl (pH 7.4) and centrifuged at 30,000 × *g* for 30 min. The sucrose-buffer interface was collected, diluted with Buffer A, and centrifuged at 30,000 × *g* for 20 min. The pellet was washed by resuspension in Buffer A and centrifugation at 30,000 × *g* for 20 min. The resulting pellet was suspended and homogenized in 50 mM Tris-HCl/6 mM $MgCl_2$/2 mM EDTA/100 mM NaCl/0.1% ascorbate/10 μM pargyline (pH 7.4 at 25°) (Buffer B) at a concentration of 3.60 ± 1.03 mg/ml (2 ml of buffer/g of tissue). The particulate preparations thus obtained were frozen in liquid nitrogen and stored at −90° until used.

Radioligand binding assay. Porcine anterior pituitary membranes (0.36 ± 0.10 mg) were incubated with 150–200 pM [^{3}H]*n*-propylapomorphine or 120–200 pM [^{3}H] spiroperidol in the presence of the indicated agents in a total assay volume of 1 ml of Buffer B for 60 min at 25°. Binding was initiated by the addition of membranes. The incubation was terminated by the addition of 5 ml of cold 2 mM $MgCl_2$/25 mM Tris-HCl (pH 7.4) (4°) buffer and rapid vacuum filtration onto Whatman GF/C or GF/B filter discs with four additional 5-ml washings. Bound radioactivity trapped on the filters was counted by liquid scintillation counting. Nonspecific binding was determined in the presence of 1 μM (+)-butaclamol.

NEM treatment. Membrane preparations were incubated with the indicated concentrations of NEM for 30 min at 25° prior to the addition of radioligand. NEM reached its maximal effect within 30 min. In separate experiments, the effect of residual NEM on subsequent ligand binding was tested by washing the membranes prior to binding, and the same results were obtained. To demonstrate the ability of the presence of antagonist and agonist to protect against the NEM effect, [^{3}H]spiroperidol and [^{3}H]*n*-propylapormorphine were incubated with the membrane preparations for 15 min at 25° prior to the addition of NEM.

Heat treatment. Anterior pituitary membranes in Buffer B without ascorbate or pargyline at 25° were added to an equal volume of buffer previously heated to 53°. After incubation for 4 min the warm membrane suspension was quickly transferred to an equal volume of ice-cold buffer. The membranes were concentrated by centrifugation and resuspended in Buffer B.

Data analysis. Direct binding assays and indirect competition binding data were analyzed by a nonlinear least-squares curve-fitting procedure using a generalized model for complex ligand-receptor systems according to the law of mass action (11, 13). The method described in the accompanying paper (8) provides estimates for the affinity of the ligands for the different states of the receptor and for the proportion of these states, and a statistical analysis comparing "goodness of fit" between one- and two-affinity state models. K_H and K_L as well as R_H and R_L are as defined in the accompanying article (8).

RESULTS

The effect of NEM on high-affinity agonist binding mimics the effect of guanine nucleotides. Pretreatment of porcine anterior pituitary membrane preparations with increasing concentrations of NEM for 30 min at 25° reduced direct agonist [^{3}H]*n*-propylapomorphine binding, with an EC_{50} of 6.9 ± 1.9 × 10^{-6} M (Fig. 1). As shown, GTP also reduced direct [^{3}H]*n*-propylapomorphine high-affinity binding to the same extent, with an EC_{50} of 1.8 × 10^{-5} M. This apparent reduction in agonist binding by NEM treatment was shown to be the result of a decrease in the number of agonist high-affinity binding sites without a significant change in the affinity of the remaining agonist high-affinity sites (Fig. 2). The dissociation constants calculated for the saturation curves in the presence of 0.2 and 10 μM NEM were 150 ± 25 pM, 297 ± 122 pM, and 153 ± 145 pM, respectively, whereas the concentrations of agonist bound were 21.8 ± 1.9 pM, 12.4 ± 3.3 pM, and 3.25 ± 1.66 pM (Fig. 2). The changes produced by NEM on direct [^{3}H]*n*-propylapomorphine

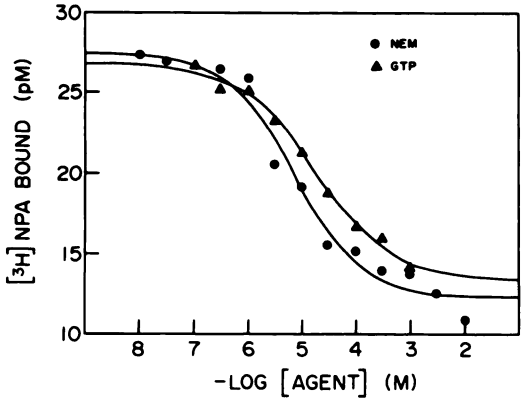

FIG. 1. *Effect of NEM treatment and the presence of GTP on direct [^{3}H]n-propylapomorphine ([^{3}H]NPA) binding*

●, Membranes were pretreated with increasing concentrations of NEM for 30 min at 25° prior to incubation with 160 pM [^{3}H]NPA. ▲, Membranes were incubated with 160 pM [^{3}H]NPA with increasing concentrations of GTP as described under Methods. (The dose-response curve for NEM treatment had an EC_{50} of 6.9 μM, whereas the curve for GTP had an EC_{50} of 18 μM.) Data shown are representative of three experiments performed in duplicate.

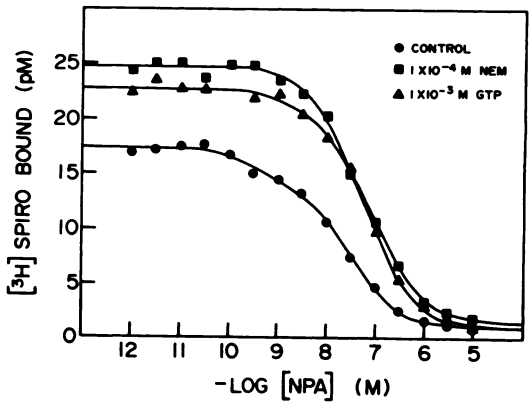

FIG. 3. *Comparison of the effects of NEM treatment and the presence of 1 mM GTP on the ability of n-propylapomorphine (agonist) to compete for [^{3}H]spiroperidol ([^{3}H]SPIRO) binding*

Membranes were incubated with [^{3}H]spiroperidol (~109 pM) for 1 hr at 25° and increasing concentrations of *n*-propylapomorphine (*NPA*). ●, Control membranes; ■, membranes treated in the presence of 100 μM NEM as described under Methods; ▲, membranes incubated in the presence of 1 mM GTP. The curves represent computer-drawn lines that best fitted the data points. All three curves are from a single experiment that was representative of three experiments.

binding are qualitatively and quantitatively the same as the effects of guanine nucleotides (cf. Fig. 4 and ref. 8).

The effect of NEM can also be demonstrated on agonist competition curves for ^{3}H-labeled antagonist binding. Figure 3 shows that pretreatment with 100 μM NEM shifts the *n*-propylapomorphine competition curve for [^{3}H]spiroperidol binding to the right and steepens the curve (Fig. 3). The same results were obtained when the experiment was performed in the presence of 1 mM GTP (Fig. 3). In addition, both NEM and GTP had the same effect on the antagonist [^{3}H]spiroperidol binding, an apparent increase in the binding at low ligand concentrations. Quantitative analysis of these data (Table 1) indicates that in the control curve an equal proportion of agonist high- and low-affinity forms (K_H = 0.27 nM and K_L = 9.7 nM) exists and that the high-affinity form is converted to the low-affinity form by both GTP and NEM, with K_L values of 14 and 18 nM, respectively. The number of total binding sites in all three curves was not significantly different (40, 35, and 32 pM).

Differential effects of NEM on agonist and antagonist binding. Figure 4 shows that the decrease in [^{3}H]*n*-propylapomorphine binding occurred at relatively low concentrations of NEM (EC_{50} = 6 μM) (Figs. 4 and 2). These concentrations did not affect [^{3}H]spiroperidol binding. However, at higher concentrations of NEM (>100 μM), [^{3}H]spiroperidol was decreased in a dose-related fashion, with an EC_{50} of 1.2 ± 0.1 mM (Fig. 4). Whereas incubation of the membranes with [^{3}H]*n*-propylapomorphine (140 pM) for 15 min prior to treatment

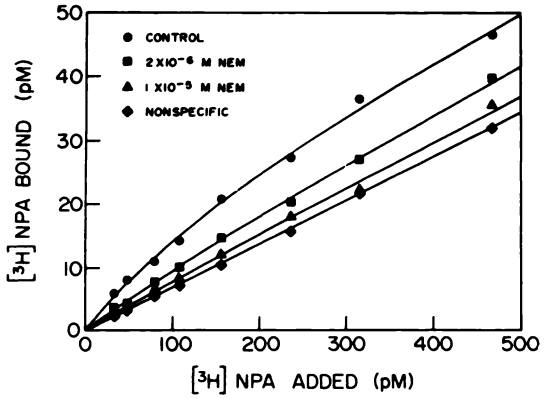

FIG. 2. *Effect of NEM treatment on direct agonist [^{3}H]-n-propylapomorphine ([^{3}H]NPA) binding*

Membranes were pretreated with none (control) (●), 2 × 10^{-6} M NEM (■), and 1 × 10^{-5} M NEM (▲) and incubated as described under Methods with increasing concentrations of [^{3}H]*n*-propylapomorphine. Nonspecific binding (◆) was determined in the presence of 1 μM (+)-butaclamol. Each point represents the average of duplicate determinations, and the experiment shown is representative of three experiments. Curves were analyzed by computer-based methods.

TABLE 1

Quantitative analysis of n-propylapomorphine competition curves for [^{3}H]spiroperidol binding in the presence of NEM and guanine nucleotide

Data from Fig. 3 were computer-analyzed as described under Methods. K_H, K_L, R_H, and R_L represent the dissociation constants for the agonist (*n*-propylapomorphine) high-affinity and low-affinity forms of the receptor and the estimated numbers of these forms of the receptor. For NEM and GTP, the single dissociaton constant correspond to the low-affinity dissociation constant of the control curve, whereas the number of receptor in the low-affinity form approximate the total number of receptors in the control curve. For the control curve, K_D values of 45 and 415 pM were used for [^{3}H]spiroperidol, whereas a single value of 45 pM was used for the other two curves.

Parameter	Control[a]	NEM, 100 μM[a]	GTP, 1 mM[a]
K_H (pM)	274 ± 146	—	—
K_L (pM)	9,700 ± 1,500	14,000 ± 1,500	18,000 ± 1,600
R_H (pM)	21.3 ± 3.5	—	—
R_L (pM)	18.7 ± 0.9	34.7 ± 0.6	32 ± 0.5

[a] Values are means ± standard error of the mean.

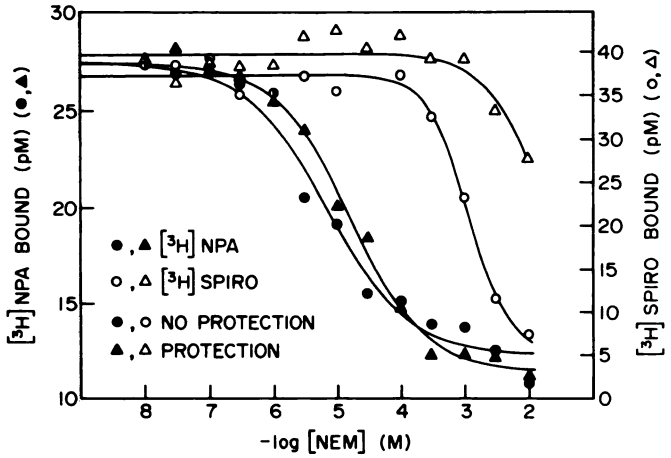

FIG. 4. *Differential sensitivity of agonist and antagonist binding to NEM treatment and ability of prior occupancy by agonist and antagonist to afford protection*

To demonstrate the ability of prior occupancy by antagonist to protect against the effect of NEM treatment on [^{3}H]spiroperidol (*[^{3}H] SPIRO*) binding, membranes were treated with NEM before (○) and after (△) the addition of 190 pM [^{3}H]spiroperidol. To examine the ability of prior occupancy by agonist to protect against the effect of NEM treatment on [^{3}H]*n*-propylapomorphine binding, membranes were treated with NEM before (●) and after (▲) the addition of 160 pM [^{3}H]*n*-propylapomorphine as described under Methods. [^{3}H]spiroperidol binding (~190 pM) and [^{3}H]*n*-propylapomorphine binding (160 pM) were performed on different membrane preparations, although each experiment is representative of two experiments carried out in duplicate.

with NEM provided little (if any) protection against the reduction in agonist high-affinity binding by NEM, the preincubation of membranes with [^{3}H]spiroperidol (150 pM) shifted the EC_{50} for NEM inhibition of antagonist binding by roughly 15- to 20-fold (Fig. 4). The apparent

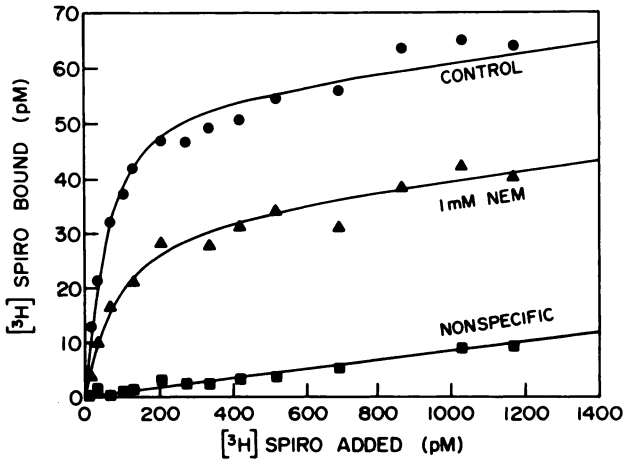

FIG. 5. *Effect of NEM treatment on direct [^{3}H]spiroperidol ([^{3}H] SPIRO) (antagonist) binding*

Control membranes (●) and membranes treated with 1 mM NEM (▲) were incubated with increasing concentrations of [^{3}H]spiroperidol. Nonspecific binding (■) was determined in the presence of 1 μM (+)-butaclamol and was the same for both control and treated membranes. Specific binding at saturation in the control curve was 55.2 ± 1.4 pM and in the NEM curve was 30.5 ± 1.0 pM. Data were analyzed by computer, and the curve represents the best fit to the data. Each point represents the result of duplicate determinations.

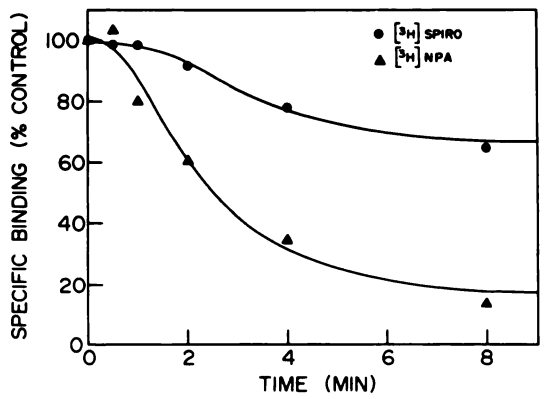

FIG. 6. *Temperature sensitivity of agonist and antagonist binding*

Porcine anterior pituitary membranes were incubated at 53° for increasing time as described under Methods. At the end of the incubation, membranes were rapidly chilled and incubated with [^{3}H]spiroperidol (*[^{3}H]SPIRO*) (~209 pM) and [^{3}H]*n*-propylapomorphine (*[^{3}H] NPA*) (~162 pM) for 1 hr at 25°; 100% binding represents 46.3 pM for [^{3}H]spiroperidol and 18 pM for [^{3}H]*n*-propylapomorphine.

reduction in antagonist binding is due to a decrease in the total number of receptors, as shown in Fig. 5. The number of receptors decreased from 55.2 ± 1.4 pM to 30.5 ± 1 pM after treatment with 1 mM NEM, whereas the affinity of the receptor for spiroperidol was unchanged (34.1 ± 4.4 pM).

Effects of heat treatment on agonist and antagonist binding. Incubating membranes at 53° preferentially decreased agonist binding, as shown in Fig. 6. After a 4-min exposure, [^{3}H]*n*-propylapomorphine binding was reduced by 65% while [^{3}H]spiroperidol binding was decreased by only 23%. This preferential reduction of agonist binding could also be demonstrated on agonist competition curves for [^{3}H]spiroperidol binding (Fig. 7). This effect of heat on agonist binding also mimics the effect of

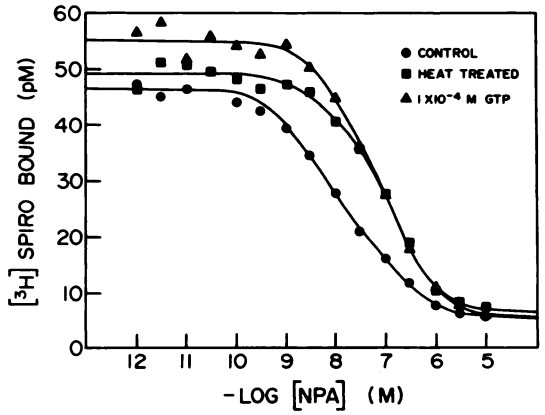

FIG. 7. *Comparison of the effect of heat treatment and guanine nucleotides on agonist competition of ^{3}H-labeled antagonist binding*

Control membranes and membranes treated at 53° for 4 min as described under Methods were incubated with [^{3}H]spiroperidol (*[^{3}H] SPIRO*) (190 pM) in the presence of increasing concentrations of *n*-propylapomorphine (*NPA*). One set of membranes was also incubated in the presence of 100 μM GTP. Data were analyzed by computer-based methods as described under Methods, and the lines through the points represent the best fit to the data. The experiment shown was performed in duplicate and is representative of two such experiments.

GTP, as shown in Fig. 7. Both the heat treatment and the presence of 100 μM GTP steepened and shifted the agonist competition to the right. The difference in the maximal binding of the *n*-propylapomorphine competition curve for [^{3}H]spiroperidol binding with the heat-treated membranes as compared with the competition curve in the presence of GTP is due to the slight sensitivity of [^{3}H]spiroperidol binding to the heat treatment (cf. Fig. 6).

DISCUSSION

The differential effects of NEM treatment on agonist and antagonist binding as indicated by the widely separated EC_{50} of these two effects indicate that two functionally different and essential sulfhydryl groups are involved in the binding of agonists and antagonists to dopamine receptor in porcine anterior pituitary membrane preparations. At micromolar concentrations of NEM, the apparent reduction in ^{3}H-labeled agonist binding appears to be due to the prevention of the formation or destabilization of the agonist high-affinity form of the receptor without a decrease in the total number of receptors. In the presence of NEM, the receptor exists virtually completely in an agonist low-affinity form as demonstrated by the *n*-propylapomorphine competition curves of [^{3}H]spiroperidol binding. The effect of NEM could also be demonstrated on direct [^{3}H]*n*-propylapomorphine binding. The apparent decrease in number of binding sites was not prevented by prior occupation of the receptor by agonist, indicating that the sulfhydryl group is not likely to be at the binding site on the receptor but at a location that interferes with the formation of the agonist high-affinity form. This interference in the formation of the agonist high-affinity form of the receptor may be caused either by preventing the receptor from forming the proper conformation needed for agonist high-affinity binding or by preventing the association of the putative guanine nucleotide regulatory protein with the agonist-receptor complex. Similar effects of NEM have been demonstrated on the ability of the *beta*-adrenergic receptor of the frog erythrocyte to form an agonist high-affinity ternary complex (13, 14). However, the exception in the frog erythrocyte *beta*-adrenergic receptor system is that formation of the agonist receptor complex prior to NEM treatment protected against the NEM effects. Therefore it appears that, like nucleotides, NEM at micromolar concentrations converts the agonist high-affinity form of the receptor into the form of the receptor possessing low affinity for the agonist *n*-propylapomorphine. NEM at micromolar concentrations also mimicked the effects of nucleotides on antagonist binding, leading to an apparent increase in [^{3}H]spiroperidol binding (Fig. 3). As shown previously (8), this can be attributed to a conversion of the antagonist low-affinity form of the receptor to a single antagonist high-affinity form in the presence of GTP.

At millimolar concentrations, NEM treatment reduced [^{3}H]spiroperidol binding. This effect was shown to be due to reduction in the number of [^{3}H]spiroperidol binding sites without a change in the affinity of the remaining sites for [^{3}H]spiroperidol. Prior occupation of the receptor by [^{3}H]spiroperidol afforded protection of the receptor from inactivation by NEM, suggesting that an essential sulfhydryl group may be located at the proximity or in the active binding site of the receptor.

Recently Suen *et al.* (17) described the effects of NEM on dopaminergic ligand binding to calf striatal membranes. At 100 μM NEM, [^{3}H]dopamine binding was reduced by 60% whereas [^{3}H]spiroperidol binding was unaffected. It was not determined whether the reduction in agonist binding was due to a decrease in the number of receptors or a change in the affinity of agonist binding. The interpretation of the results was complicated by the fact that the striatum contains at least two separate, and maybe more, dopamine receptor subtypes to which dopamine could bind with high affinity (18).

We have shown that the heat treatment of porcine anterior pituitary membrane preparations at 53° for 4 min preferentially reduces direct [^{3}H]*n*-propylapomorphine binding and that, on agonist competition curves for [^{3}H]spiroperidol binding, heat treatment mimics the effect of guanine nucleotides by reducing the ability to see the agonist high-affinity form of the receptor. However, with the same treatment, no major loss in the total number of receptors that are labeled with [^{3}H]spiroperidol occurs. In a similar manner, Lew and Goldstein (19) reported that the potencies of dopaminergic agonists to displace [^{3}H]spiroperidol binding as well as [^{3}H]dopamine binding were significantly reduced in thermally exposed striatal membranes whereas the potencies of dopaminergic antagonists were not altered. Their interpretation that dopamine receptors with high affinities for agonists and antagonists may represent two distinct molecular entities or two different binding sites on the same receptor molecule having thermally different stabilities is complicated by the fact that multiple subtypes of dopamine receptors in brain can bind [^{3}H]dopamine (18). In the studies reported here, the qualitative and quantitative aspects of the effects of NEM and heat treatments on agonist and antagonist binding are consistent with a selective inactivation of the putative guanine nucleotide regulatory protein presumably associated with the agonist high-affinity/antagonist low-affinity form of the receptor.

The similar sensitivities of the [^{3}H]spiroperidol binding sites in striatal membranes and in porcine anterior pituitary membranes to guanine nucleotides, NEM treatment, and heat treatment suggest that functionally and structurally these two dopaminergic receptors may be similar. The fact that radically different treatments—guanine nucleotides, NEM, and heat—produce essentially the same qualitative and quantitative effects on agonist high-affinity binding clearly suggests that they may be evoked through a common component involved in the formation of the agonist high-affinity form of the receptor. Because of the specificity of the nucleotide effects, it is reasonable to suggest the involvement of a guanine nucleotide-binding protein in this process. The guanine nucleotide regulatory protein involved in the agonist high-affinity state of the *beta*-adrenergic receptor is sensitive to NEM treatment (14). However, these effects on the *beta*-adrenergic receptor system take place in the range of millimolar concentrations of NEM. The greater sensitivity of the dopaminergic agonist high-affinity form of the receptor might reflect differences in the two systems. The *beta*-adrenergic receptor is coupled to a stimulation of ade-

nylate cyclase, whereas the dopaminergic receptor of the pituitary gland appears to attenuate adenylate cyclase activity. It has been suggested that the guanine nucleotide regulatory protein involved in stimulatory systems may be different from the functional entity postulated to mediate nucleotide effects on inhibitory systems such as the *alpha*$_2$-adrenergic receptor (1). Of interest is the observation that NEM at micromolar concentrations inhibits the *alpha*$_2$-adrenergic attenuation of adenylate cyclase.[3] A nucleotide-sensitive agonist high-affinity form of the receptor is also present in this system (20, 21).

The temperature susceptibility of the agonist high-affinity form of the dopaminergic receptor of the porcine anterior pituitary gland as reported here is very similar to the heat sensitivity of the functional nucleotide-binding component implicated in the stimulation of adenylate cyclase by *beta*-adrenergic drugs or nucleotides and fluoride ions in the S-49 lymphoma system (15). These data on the effects of nucleotides, NEM, and heat treatments on dopaminergic agonist and antagonist binding and their striking similarities with other receptor systems suggest that the dopaminergic agonist high-affinity form of the receptor and reciprocally the antagonist low-affinity form (8) result from the interaction of a guanine nucleotide regulatory protein with the receptor. Further biochemical studies are needed to document a direct association of both molecular entities.

ACKNOWLEDGMENTS

We thank Ms. Lynn Tilley and Ms. Donna Addison for preparation of the manuscript.

REFERENCES

1. Rodbell, M. The role of hormone receptors and GTP-regulatory proteins in membrane transduction. *Nature* (*Lond.*) **284**:17–22 (1980).
2. Zahniser, N. R., and P. B. Molinoff. Effect of guanine nucleotides on striatial dopamine receptors. *Nature* (*Lond.*) **275**:453–455 (1978).
3. Goldstein, M., A. Leiberman, J. Y. Lew, T. Asano, M. R. Rosenfeld, and M. H. Makman. Interactions of pergolide with central dopaminergic receptors. *Proc. Natl. Acad. Sci. U. S. A.* **77**:3725–3728 (1980).
4. Creese, I., T. B. Usdin, and S. H. Snyder. Dopamine receptor binding regulated by guanine nucleotides. *Mol. Pharmacol.* **16**:69–76 (1979).
5. Sibley, D. R., and I. Creese. Guanine nucleotides regulate anterior pituitary dopamine receptors. *Eur. J. Pharmacol.* **55**:341–343, (1979).
6. Caron, M. G. Guanine nucleotides modulate the affinity of the dopamine receptor for agonists in bovine anterior pituitary. *Proc. 61st Annual Meeting Endocr. Soc.*, June 13–15, Anaheim, Calif., 81 (1979).
7. Sibley, D. R. and I. Creese. Dopamine receptor binding in bovine intermediate pituitary membranes. *Endocrinology* **107**:1405–1409 (1980).
8. De Lean, A., B. F. Kilpatrick, and M. G. Caron. Dopamine receptor of the porcine anterior pituitary gland: evidence for two affinity states of the receptor discriminated by both agonists and antagonists. *Mol. Pharmacol.* **22**:290–297 (1982).
9. Maguire, M. E., P. M. Van Arsdale, and A. G. Gilman. An agonist-specific effect of guanine nucleotides on binding to the *beta*-adrenergic receptor. *Mol. Pharmacol.* **12**:335–339 (1976).
10. Lefkowitz, R. J., D. Mullikin, and M. G. Caron. Regulation of β-adrenergic receptors by guanyl-5′-yl imidophosphate and other purine nucleotides. *J. Biol. Chem.* **251**:4686–4692 (1976).
11. Kent, R. S., A. De Lean, and R. J. Lefkowitz. A quantitative analysis of *beta*-adrenergic receptor interactions: resolution of high and low affinity states of the receptor by computer modeling of ligand binding data. *Mol. Pharmacol.* **17**:14–23 (1980).
12. Limbird, L. E., D. M. Gill, and R. J. Lefkowitz. Agonist promoted coupling of the beta-adrenergic receptor with the guanine nucleotide regulatory protein. *Proc. Natl. Acad. Sci. U. S. A.* **77**:775–779 (1980).
13. De Lean, A., J. M. Stadel, and R. J. Lefkowitz. A ternary complex model explains the agonist-specific binding properties of adenylate-cyclase coupled beta-adrenergic receptor. *J. Biol. Chem.* **255**:7108–7117 (1980).
14. Stadel, J. M., and R. J. Lefkowitz. Multiple reactive sulfhydryl groups modulate the function of adenylate cyclase coupled *beta*-adrenergic receptors. *Mol. Pharmacol.* **16**:709–718 (1979).
15. Ross, E. M., and A. G. Gilman. Resolution of some components of adenylate cyclase necessary for catalytic activity. *J. Biol. Chem.* **252**:6966–6969 (1979).
16. Stadel, J. M., R. G. L. Shorr, L. E. Limbird, and R. J. Lefkowitz. Evidence that a β-adrenergic receptor-associated guanine nucleotide regulatory protein conveys guanosine 5′-*O*-(3-thiotriphosphate)-dependent adenylate cyclase activity. *J. Biol. Chem.* **256**:8718–8723 (1981).
17. Suen, E. T., E. Stefanini, and Y. C. Clement-Cormier. Evidence for essential thiol groups and disulfhyde bonds in agonists and antagonists binding to the dopamine receptor. *Biochem. Biophys. Res. Commun.* **96**:953–960 (1980).
18. Seeman, P. Brain dopamine receptors. *Pharmacol. Rev.* **32**:229–313 (1981).
19. Lew, J. Y., and M. Goldstein. Dopamine receptor binding for agonists and antagonists in thermally exposed membranes. *Eur. J. Pharmacol.* **55**:429–430 (1979).
20. Hoffman, B. B., D. Mullikin-Kilpatrick, and R. J. Lefkowitz. Heterogeneity of radioligand binding to α-adrenergic receptors: analysis of guanine nucleotide regulation in relation to receptor subtypes. *J. Biol. Chem.* **255**:4645–4652 (1980).
21. Michel, T., B. B. Hoffman, R. J. Lefkowitz, and M. G. Caron. Different sedimentation properties of agonist and antagonist-labelled alpha$_2$-adrenergic receptors. *Biochem. Biophys. Res. Commun.* **100**:1131–1136 (1981).

[3] T. Michel and R. J. Lefkowitz, personal communication.

Send reprint requests to: Dr. Marc G. Caron, Box 3287, Duke University Medical Center, Durham, N. C. 27710.