Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 3906–3910, May 1989
Neurobiology

Corticosterone differentially regulates the expression of $G_{s\alpha}$ and $G_{i\alpha}$ messenger RNA and protein in rat cerebral cortex

(G proteins/glucocorticoids/adrenalectomy)

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Communicated by Aaron B. Lerner, February 21, 1989 (received for review October 24, 1988)

ABSTRACT The possibility that glucocorticoids regulate specific guanine nucleotide binding regulatory proteins (G proteins) was investigated in rat cerebral cortex. Corticosterone was administered to normal and bilaterally adrenalectomized rats, and hormone regulation of individual G-protein subunits was investigated in cerebral cortex in three ways: (*i*) immunoblot analysis of subunit protein, (*ii*) hybridization blot analysis of subunit mRNA, and (*iii*) ADP-ribosylation analysis of stimulatory G protein ($G_{s\alpha}$) subunits. Chronic (7 days) corticosterone administration to normal rats increased levels of $G_{s\alpha}$ immunoreactivity, mRNA, and ADP-ribosylation but decreased levels of inhibitory G protein ($G_{i\alpha}$) mRNA and tended to decrease levels of $G_{i\alpha}$ immunoreactivity. In contrast, levels of $G_{o\alpha}$ and G_β immunoreactivity and mRNA were not influenced by corticosterone treatment. In adrenalectomized rats, corticosterone treatment produced a 25–50% increase in the levels of $G_{s\alpha}$ immunoreactivity, mRNA, and ADP-ribosylation, whereas the hormone produced a 20–35% decrease in the levels of $G_{i\alpha}$ immunoreactivity and mRNA. Adrenalectomy, without corticosterone replacement, produced the opposite effects on $G_{s\alpha}$ and $G_{i\alpha}$ compared to sham-operated controls, indicating that these G proteins are regulated by this class of steroid hormone under physiological conditions *in vivo*. The results indicate that specific G-protein subunits—namely, $G_{s\alpha}$ and $G_{i\alpha}$—are under the coordinated control of glucocorticoids in rat brain and demonstrate that G proteins are physiological targets of glucocorticoids *in vivo*. Possible roles played by these G-protein responses in mediating the effects of glucocorticoids on brain function are discussed.

Glucocorticoids produce profound behavioral, physiological, and biochemical effects in brain (1, 2). Actions of the hormone in the nervous system are thought to be achieved in large part through the regulation of gene expression and protein synthesis in target cells, as has been described in nonnervous tissues (3). Prominent among the known targets of glucocorticoid action in brain are some of the individual proteins that comprise signal transduction pathways, including neurotransmitter synthetic enzymes and second messenger and protein phosphorylation systems (1, 2, 4–11). In this study, we have investigated the possibility that G proteins, a family of GTP-binding proteins that appear to play a central role in coupling neurotransmitter receptors to numerous types of intracellular effector systems, are also targets of glucocorticoid action in the central nervous system.

Three major classes of G proteins have been characterized in brain: G_s, G_i, and G_o. It is generally thought that G_s and G_i, respectively, mediate the activation and inhibition of adenylate cyclase by stimulatory and inhibitory neurotransmitters, whereas G_o (as well as some forms of G_i) may mediate the actions of neurotransmitters on other effector systems, such as ion channels and phospholipases (12–14). G proteins are heterotrimers composed of single α, β, and γ subunits. Distinct α subunits confer specific functional activity on the different types of G proteins, which appear to share common $\beta\gamma$ subunits. Individual G-protein subunits have been characterized extensively at the biochemical and molecular level (see refs. 12, 13, and 15). Two species of $G_{s\alpha}$ subunits are prominent in brain: 52- and 45-kDa proteins possibly derived from alternate splicing of a single gene. At least three species of $G_{i\alpha}$ subunits are also prominent in brain: 40- to 41-kDa proteins derived apparently from separate genes (15–17). Only one species of $G_{o\alpha}$ subunit is known in brain: a 39-kDa protein. Two forms of G_β are known: 36- and 35-kDa proteins derived apparently from distinct genes, whereas less is known about several variant forms of G_γ, which awaits further characterization.

In the present study, we utilized antisera and cDNA probes specific for these various G-protein subunits to study whether one or more of the individual subunits is under the control of glucocorticoids in rat brain. We report here that glucocorticoid administration increases levels of $G_{s\alpha}$ protein and mRNA but decreases levels of $G_{i\alpha}$ protein and mRNA in rat cerebral cortex. The results raise the possibility that some of the effects of glucocorticoids on brain function are achieved through the specific regulation of these G-protein subunits.

METHODS

***In Vivo* Drug Treatments.** Male Sprague–Dawley rats (initial weight, 175–225 g) were implanted subcutaneously with a single pellet containing 100 mg of corticosterone in a cholesterol base (Innovative Research of America) under light halothane anesthesia. Such corticosterone treatment has been shown to maintain a physiological serum concentration of corticosterone for at least 14 days (18). Control rats either underwent identical surgery with implantation of cholesterol pellets (Innovative Research) or underwent no treatment. These two types of controls did not differ in final determinations of G-protein levels. Unless specified otherwise, rats were used 7 days after pellet implantations.

In other experiments, rats underwent bilateral adrenalectomy under halothane anesthesia. Some adrenalectomized rats were implanted subcutaneously with a single corticosterone pellet immediately after adrenalectomy. Control animals were sham-operated; adrenal glands were identified but not excised. Animals were given free access to normal drinking water and 0.9% saline and were used 7 days after surgery.

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Abbreviations: G protein, guanine nucleotide binding regulatory protein; G_s, stimulatory G protein; G_i, inhibitory G protein.
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Quantitation of G-Protein Immunoreactivity. Brains were removed rapidly from decapitated rats and cerebral cortex was isolated by gross dissection. The tissue was homogenized (20 mg/ml) in glass/Teflon homogenizers in an ice-cold solution of 40 mM Tris·HCl, pH 6.8/1 mM dithiothreitol/2% $NaDodSO_4$, and the homogenates were frozen and stored for up to 2 weeks. Aliquots of the homogenates were used for protein determinations by the method of Lowry *et al.* (19) using bovine serum albumin as a standard. Other aliquots of the homogenates were used for G-protein immunoblots as described (20).

Briefly, duplicate aliquots (containing 20–40 μg of protein), adjusted to a vol of 30 μl with homogenization buffer, were incubated in a 75°C water bath for 5 min, after which time 20 μl of 100 mM *N*-ethylmaleimide was added, and the mixtures were incubated for an additional 15 min at room temperature. Next, 50 μl of a solution containing 40 mM Tris·HCl, pH 6.8, 2% $NaDodSO_4$, 40% (vol/vol) glycerol, 6% 2-mercaptoethanol was added and the mixtures were boiled for 2 min. The samples were then subjected to $NaDodSO_4$/polacrylamide gel electrophoresis as described (21) with 9% acrylamide/0.25% bisacrylamide in the resolving gels. Proteins in resulting gels were transferred electrophoretically to nitrocellulose papers, which were then immunolabeled for specific G-protein subunits using rabbit polyclonal antisera for $G_{s\alpha}$, $G_{i\alpha}$, $G_{o\alpha}$, or G_β and ^{125}I-labeled goat anti-rabbit IgG (500 cpm/μl; New England Nuclear) exactly as described (22). $G_{s\alpha}$ antiserum [no. 584, which recognizes only the 45-kDa form of the protein in crude tissue extracts (ref. 12; see below)] was provided by A. G. Gilman (University of Texas at Dallas); $G_{i\alpha}$ antiserum [no. AS/7, which recognizes two (41 and 40 kDa) forms of $G_{i\alpha}$ in brain (16)] was provided by A. Spiegel (National Institutes of Health); and $G_{o\alpha}$ antiserum and G_β antiserum (which recognizes both the 36- and 35-kDa forms of the β subunit) were provided by J. Northup (Yale University). Immunolabeled G-protein bands were identified by autoradiography and cut out of dried blots, and radioactivity was then quantitated by a γ-counter.

Under the immunoblotting conditions used and for each of the antisera used, levels of immunolabeling of the G-protein subunits were linear over at least a 3-fold range of tissue protein loaded on the original gels. In addition, for each of the antisera used except that for $G_{i\alpha}$, levels of G-protein immunoreactivity in brain samples were compared to immunoreactivity levels for purified G-protein subunits included in the same gels. [Purified G-protein subunits were kindly provided by J. Northup (Yale University).] It was found that immunolabeling of the purified subunits was linear over a 5- to 10-fold concentration range and that mixtures of brain samples and purified subunits led to additive levels of immunoreactivity. These experiments confirmed that the $G_{s\alpha}$ antiserum used in this study showed a severalfold preference for the 45-kDa form of the protein: the antiserum barely detected the 52-kDa form in either purified protein or crude tissue preparations (see also ref. 23). Based on the immunolabeling of purified G-protein subunits, levels of $G_{s\alpha}$ (45-kDa form only), $G_{o\alpha}$, and G_β (35- and 36-kDa forms) in cerebral cortex from control rats were, respectively, 1.0, 14.9, and 10.4 ng per μg of protein (means of duplicate determinations in three rats). Similar quantitation could not be achieved for $G_{i\alpha}$ because of the unavailability of this purified G-protein subunit and because of its considerable heterogeneity. However, similar control experiments with the $G_{i\alpha}$ antiserum used in the present study have been reported (16).

Quantitation of G-Protein mRNA Levels. Samples of cerebral cortex (sometimes from the same animals used for immunolabeling experiments) were homogenized (1 g per 4 ml) in ice-cold 4 M guanidine isothiocyanate/25 mM sodium acetate, pH 6/0.8% 2-mercaptoethanol, and total RNA was isolated by CsCl centrifugation as described (24). Aliquots (containing 2–10 μg of total RNA as estimated by UV absorbance) were subjected to hybridization blot analysis exactly as described (24) using cDNA clones generously provided by R. Reed (Johns Hopkins University). $G_{s\alpha}$, $G_{i\alpha1}$, $G_{i\alpha2}$, and $G_{o\alpha}$ cDNAs were cloned into the GEM vector and G_β cDNA was cloned into the Bluescript(−) vector, all at the R1 site (15). The cDNA clones were nick-translated by standard procedures (see ref. 24) and hybridizations were conducted with 2–5 × 10^7 cpm of [^{32}P]cDNA clones per blot. Quantitation of hybridization was carried out by densitometry of resulting autoradiographs; although such quantitation cannot be related to absolute values of mRNA, it is valid and reproducible when data obtained from a single experiment and on the same blot are being analyzed.

The amount of total RNA applied to the gels was quantitated by hybridizing the same blots with ^{32}P-labeled oligo(dT) as described (25). Oligo(dT) (20-mer; Pharmacia) was end-labeled with T4 polynucleotide kinase by a standard procedure (see ref. 24). Nitrocellulose filters were stripped of G-protein cDNA by boiling them for 5 min in water; they were then blotted with 40 pmol of oligo(dT) per 40 cm^2 of filter paper as described above. Levels of oligo(dT) bound were quantitated by liquid scintillation spectrometry of excised lanes. Levels of G-protein mRNA were calculated as G-protein blotting divided by oligo(dT) blotting. Under the blotting conditions used, levels of G-protein mRNA were linear over a 3-fold range of total RNA concentration, and the effects of corticosterone and adrenalectomy on $G_{s\alpha}$ and $G_{i\alpha}$ were observed over this entire range.

Quantitation of $G_{s\alpha}$ ADP-Ribosylation. Cerebral cortex was homogenized (10 mg/ml) in an ice-cold solution of 50 mM Tris·HCl, pH 7.4/1 mM EDTA/2 mM $MgCl_2$/10% sucrose, and ADP-ribosylation of $G_{s\alpha}$ was carried out on crude particulate fractions exactly as described (26) using purified cholera toxin α subunit (List Biochemical Laboratories, Campbell, CA) and [^{32}P]NADP (≈30 Ci per mmol; 1 Ci = 37 GBq; New England Nuclear). The samples were subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis as described above. ^{32}P-labeled $G_{s\alpha}$ subunits were identified by autoradiography and individual bands were cut out of dried gels and quantitated by liquid scintillation spectrometry; alternatively, densitometry was used in some experiments for quantitation, which yielded similar results. Under the assay conditions used, ADP-ribosylation levels of $G_{s\alpha}$ were linear over a 3-fold range of tissue concentration, and the effect of corticosterone on ADP-ribosylation levels was observed over this entire range. Levels of cholera toxin-mediated ADP-ribosylation of $G_{s\alpha}$ obtained by this method (17.5 pmol per mg of membrane protein) are similar to those reported (26). It was found that such ADP-ribosylation levels were >85% of levels obtained when greater amounts of cholera toxin, higher NAD concentrations, and longer incubation times were used.

RESULTS

Effect of Corticosterone on Levels of G-Protein Immunoreactivity. Rats were treated with corticosterone for 1 week, conditions known to elicit a number of behavioral, physiological, and biochemical responses to the hormone (see refs. 1 and 2), and levels of G-protein immunoreactivity were quantitated in cerebral cortex by immunoblotting procedures. It was found (Fig. 1 and Table 1) that corticosterone produced a 40% increase in levels of the 45-kDa form of $G_{s\alpha}$ and appeared to produce a small (≈15%) decrease in $G_{i\alpha}$ (although this latter change was reproducible, it was not statistically significant). In contrast, corticosterone did not alter levels of $G_{o\alpha}$ and G_β immunoreactivity.

The increase observed in the 45-kDa form of $G_{s\alpha}$ was time dependent in that it required chronic exposure to corticoste-

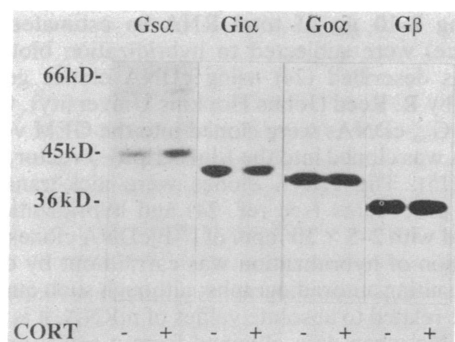

FIG. 1. Autoradiograms showing the effect of corticosterone on levels of G-protein immunoreactivity. Rats were treated with corticosterone for 7 days, after which time cerebral cortex was isolated and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis. Resulting gels were then subjected to immunoblot analysis with antisera directed against $G_{s\alpha}$, $G_{i\alpha}$, $G_{o\alpha}$, or G_β and with ^{125}I-labeled goat anti-rabbit IgG; autoradiograms were obtained from resulting blots. CORT, corticosterone.

rone. Levels of $G_{s\alpha}$ were unaltered after 1 day of hormone treatment and showed a small, but statistically significant, increase after 4 days of treatment (data not shown). These shorter treatment times did not produce detectable changes in the other G-protein subunits.

Effect of Corticosterone on Levels of G-Protein mRNA. To determine whether changes in $G_{s\alpha}$, and possibly in $G_{i\alpha}$, immunoreactivity can be related to changes in their mRNAs, the effect of corticosterone on levels of G-protein mRNA was studied by hybridization (Northern) blot procedures, as described in *Methods*. One week of corticosterone exposure was found to produce a >30% increase in mRNA levels of $G_{s\alpha}$ and a 25% decrease in mRNA levels of $G_{i\alpha}$ in cerebral cortex (Fig. 2 and Table 1). In contrast, no changes were observed in mRNA levels for $G_{o\alpha}$ or G_β. One day of exposure to corticosterone was without effect on mRNA levels for the various G-protein subunits (data not shown), indicating that the changes in $G_{s\alpha}$ and $G_{i\alpha}$ mRNA are dependent on chronic exposure to the hormone.

The data reported for $G_{i\alpha}$ in Fig. 2 and Table 1 reflect levels of $G_{i\alpha2}$ mRNA. Analogous experiments performed with a cDNA probe specific for $G_{i\alpha1}$ produced equivalent results. Thus, levels of $G_{i\alpha1}$ mRNA were decreased by chronic corticosterone administration (data not shown).

Effect of Adrenalectomy on Levels of G-Protein mRNA and Immunoreactivity. Regulation by corticosterone of $G_{s\alpha}$ and $G_{i\alpha}$ protein and mRNA levels indicates that these G-protein subunits can be altered by exogenous glucocorticoids. To determine whether they are also regulated by endogenous glucocorticoids *in vivo*, the effect of adrenalectomy, with and without corticosterone replacement, was studied. Bilateral adrenalectomy produced a significant (≈20%) decrease in $G_{s\alpha}$ mRNA and a significant (≈50%) increase in $G_{i\alpha}$ mRNA

Table 1. Effect of corticosterone on levels of G-protein immunoreactivity and mRNA

	% of control ± SEM (n)	
	Immunoreactivity	mRNA
$G_{s\alpha}$	141 ± 6 (5)*	133 ± 5 (9)*
$G_{i\alpha}$	85 ± 9 (5)†	76 ± 5 (8)*
$G_{o\alpha}$	99 ± 4 (6)	97 ± 7 (8)
G_β	105 ± 6 (5)	104 ± 11 (7)

Rats were treated with corticosterone for 7 days, after which time levels of G-protein immunoreactivity and mRNA were quantitated as described in *Methods*.
*$P < 0.05$ by χ^2 test.
†$P < 0.1$ by χ^2 test.

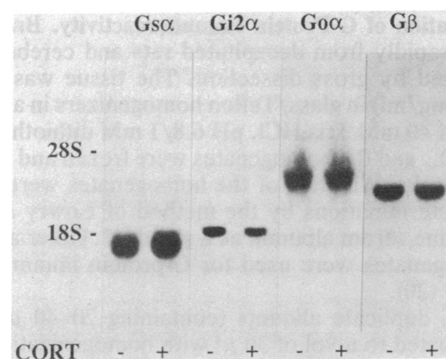

FIG. 2. Autoradiograms showing the effect of corticosterone on levels of G-protein mRNA. Rats were treated with corticosterone for 7 days, after which time total RNA extracted from isolated cerebral cortex was subjected to electrophoresis in agarose/formaldehyde gels. Resulting gels were then subjected to hybridization blot analysis with [^{32}P]cDNA probes for $G_{s\alpha}$, $G_{i\alpha1}$, $G_{i\alpha2}$, $G_{o\alpha}$, or G_β; autoradiograms were obtained from resulting blots. Only results for $G_{i\alpha2}$ mRNA are shown; similar results were obtained for $G_{i\alpha1}$ mRNA. CORT, corticosterone.

in cerebral cortex compared to mRNA levels in sham-operated control rats (Fig. 3). These effects are opposite those observed in response to corticosterone treatment of normal (unoperated) rats (Fig. 1 and Table 1). As shown in Fig. 3, the effects of adrenalectomy on $G_{s\alpha}$ and $G_{i\alpha}$ mRNA were reversed completely by corticosterone replacement.

The effect of adrenalectomy on levels of G-protein immunoreactivity was also investigated. It was found that corticosterone replacement of adrenalectomized rats significantly increased levels of $G_{s\alpha}$ immunoreactivity and significantly decreased levels of $G_{i\alpha}$ immunoreactivity in cerebral cortex compared to levels in adrenalectomized animals without corticosterone replacement (data not shown).

Effect of Corticosterone and Adrenalectomy on $G_{s\alpha}$ ADP-Ribosylation. The anti-$G_{s\alpha}$ antiserum used in this study recognizes only one of two known forms of $G_{s\alpha}$—namely, the 45-kDa form, and not the 52-kDa form, of the protein. Therefore, to assess the state of the 52-kDa form of $G_{s\alpha}$,

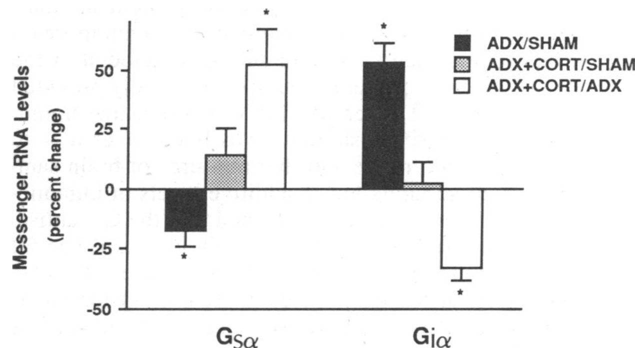

FIG. 3. Effect of adrenalectomy and corticosterone replacement on levels of G-protein mRNA. Rats were treated in one of three ways: ADX, bilateral adrenalectomy; SHAM, similar anesthesia and surgery but without adrenalectomy; or ADX+CORT, adrenalectomized and implanted with a single corticosterone pellet. One week after surgery, total RNA extracted from isolated cerebral cortex was subjected to electrophoresis in agarose/formaldehyde gels. Resulting gels were blotted with [^{32}P]cDNA probes for $G_{s\alpha}$ or $G_{i\alpha2}$; autoradiograms were obtained from the final hybridization blots. Levels of G-protein mRNA were quantitated by densitometry. The same blots were then labeled with ^{32}P-labeled oligo(dT), which was quantitated by liquid scintillation spectrometry. Data were calculated as levels of G-protein mRNA ÷ levels of oligo(dT) labeling and are expressed as percent change ± SEM. The number of rats used ranged from 4 to 11 in each treatment group.

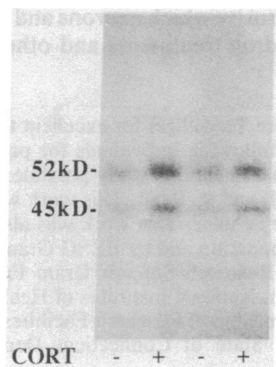

FIG. 4. Autoradiogram showing the effect of corticosterone on $G_{s\alpha}$ ADP-ribosylation levels. Rats were treated with corticosterone for 7 days, after which time isolated cerebral cortex was subjected to *in vitro* ADP-ribosylation with cholera toxin and [^{32}P]NADP and to $NaDodSO_4$/polyacrylamide gel electrophoresis; autoradiograms were obtained from resulting gels. CORT, corticosterone.

ADP-ribosylation procedures were used. Exposure of rats to corticosterone for 1 week led to significant increases in cholera toxin-mediated ADP-ribosylation of both the 45- and 52-kDa forms of $G_{s\alpha}$ in cerebral cortex (Fig. 4 and Table 2).

The effect of adrenalectomy on ADP-ribosylation levels of $G_{s\alpha}$ in cerebral cortex was also studied. Bilateral adrenalectomy was found to significantly decrease ADP-ribosylation levels of both the 45- and 52-kDa forms of $G_{s\alpha}$ compared to sham-operated controls (Table 2). This effect of adrenalectomy was reversed completely by corticosterone replacement, which resulted in levels of $G_{s\alpha}$ ADP-ribosylation about the same as those found in sham-operated controls and 30–40% higher than those found in adrenalectomized rats without corticosterone replacement (Table 2).

Regulation by corticosterone of ADP-ribosylation levels of $G_{s\alpha}$ in cerebral cortex did not appear to be due to kinetics of the ADP-ribosylation reaction. Thus, the corticosterone-induced increase in $G_{s\alpha}$ ADP-ribosylation levels was observed over a 20-fold range of NAD concentration (5–100 μM), as well as over a severalfold range of tissue concentration, cholera toxin concentration (10–50 ng/μl), and time of *in vitro* ADP-ribosylation (30 min to 2 hr) (data not shown).

DISCUSSION

This paper reports that the expression of specific G-protein subunits is under the regulatory control of glucocorticoids in the nervous system *in vivo*. We have demonstrated that glucocorticoids regulate the α subunits of two types of G protein, G_s and G_i, in this brain region and showed that such regulation by glucocorticoids occurs under physiological conditions *in vivo*. In contrast, the α subunit of a third type of G protein, G_o, as well as the β subunits thought to be common to all three types of G protein were not regulated by glucocorticoids.

Table 2. Effect of corticosterone and adrenalectomy on ADP-ribosylation levels of $G_{s\alpha}$

	ADP-ribosylation levels, % ± SEM (*n*)		
	CORT vs. control	ADX vs. sham	ADX + CORT vs. ADX
45 kDa	152 ± 8 (6)*	69 ± 2 (4)*	127 ± 11 (6)*
52 kDa	125 ± 7 (6)*	67 ± 4 (4)*	140 ± 15 (6)*
45 + 52 kDa	137 ± 8 (6)*	67 ± 3 (4)*	133 ± 13 (6)*

Normal (control) or adrenalectomized (ADX) rats were treated with corticosterone (CORT) for 7 days, after which time ADP-ribosylation levels of $G_{s\alpha}$ were determined in cerebral cortex as described in *Methods* and in Fig. 4. CORT vs. control, corticosterone treatment of control rats expressed as percent of control; ADX vs. sham, adrenalectomized rats expressed as percent of sham-operated rats; ADX + CORT vs. ADX, corticosterone treatment of adrenalectomized rats expressed as percent of adrenalectomized rats.
*$P < 0.05$ by χ^2 test.

Glucocorticoids regulate $G_{s\alpha}$ and $G_{i\alpha}$ by altering levels of the total amounts of these proteins and of their mRNA. Thus, corticosterone was shown to increase $G_{s\alpha}$ but to decrease $G_{i\alpha}$ immunoreactivity and mRNA, whereas bilateral adrenalectomy produced the opposite effects. For both G-protein subunits, changes in levels of immunoreactivity appeared to correlate temporally with changes in mRNA in that both required chronic (7 days) corticosterone exposure to become manifest and were not observed in response to shorter treatment times. Furthermore, the findings that adrenalectomy results in changes in $G_{s\alpha}$ and $G_{i\alpha}$ immunoreactivity and mRNA, and that such effects are reversed completely by corticosterone, suggest that glucocorticoids play a role in maintaining the levels of these two G-protein α subunits in brain under normal physiological conditions.

The results of the present study indicate that individual G-protein α subunits can be regulated independently in the central nervous system. Glucocorticoid regulation of both $G_{s\alpha}$ and $G_{i\alpha}$ protein and mRNA may occur through the regulation of gene expression. According to this scheme, glucocorticoids would alter the levels of mRNA for these G-protein α subunits by altering their rates of transcription. Such glucocorticoid regulation of G-protein expression could represent a direct action of glucocorticoids on G-protein genes, a possibility supported by the fact that we have identified some glucocorticoid response elements in genomic clones of $G_{s\alpha}$ and $G_{i\alpha}$. Alternatively, glucocorticoid regulation of G-protein expression could represent indirect actions of the hormone mediated through other glucocorticoid-induced proteins that would then regulate G-protein transcription. In support of this latter possibility is the observation that glucocorticoid induction of $G_{s\alpha}$ mRNA in GH_3 cultured cells is blocked by protein synthesis inhibitors, suggesting that steroid hormone regulation of $G_{s\alpha}$ in this cell type is mediated by other newly synthesized (glucocorticoid induced) proteins (27). However, it is important to note the additional possibility that the changes in steady-state levels of $G_{s\alpha}$ and $G_{i\alpha}$ mRNA and protein observed in the present study could result from changes in the turnover of mRNA and/or protein and not from the regulation of gene transcription *per se*.

The antiserum used to quantitate levels of $G_{s\alpha}$ immunoreactivity recognizes only the 45-kDa form of the protein in crude brain extracts, so the 52-kDa form could not be studied by immunoblotting procedures. However, as an indirect measure of the levels of this protein, the effect of glucocorticoids on cholera toxin-mediated ADP-ribosylation of both $G_{s\alpha}$ forms was studied. It was found that corticosterone and adrenalectomy produced similar changes in the 45-kDa protein by ADP-ribosylation and immunoblotting procedures (compare Tables 1 and 2) and that similar changes were observed in the 52-kDa protein by ADP-ribosylation procedures (Table 2). These results suggest that the two forms of $G_{s\alpha}$ are similarly regulated by glucocorticoids. However, this interpretation must be viewed with caution [since differences in ADP-ribosylation can reflect differences in properties of G proteins other than their total amounts (see refs. 12, 13, and 20)] and awaits direct confirmation made possible by the availability of antisera that recognize the 52-kDa form of $G_{s\alpha}$ in crude brain extracts.

The antiserum used for quantitation for $G_{i\alpha}$ has been shown to recognize 41- and 40-kDa forms of the protein (16) and, in some experiments in the present study, $G_{i\alpha}$ immunoblots revealed a doublet of 40–41 kDa. In those experiments in which doublets were obtained, corticosterone appeared to produce equivalent changes in both proteins, although their close migration made it impossible to directly quantitate

these changes. Hybridization blot experiments indicated that two forms of $G_{i\alpha}$ mRNA, designated $G_{i\alpha1}$ and $G_{i\alpha2}$, are regulated in similar fashion by corticosterone and adrenalectomy. Such parallel regulation is interesting in view of the fact that the variant forms of $G_{i\alpha}$ appear to represent distinct gene products (15–17). While the 41-kDa protein appears to be the product of $G_{i\alpha1}$ mRNA, the relationship between the other forms of $G_{i\alpha}$ protein and mRNA remains unknown.

Regulation of $G_{s\alpha}$ and $G_{i\alpha}$ by corticosterone and adrenalectomy in cerebral cortex has important implications concerning the molecular mechanisms by which this steroid hormone alters brain function. Up-regulation of $G_{s\alpha}$, with no change in G_{β}, might be expected to enhance basal adenylate cyclase activity, as well as to increase the ability of stimulatory neurotransmitters to activate the enzyme, as has been observed with purified $G_{s\alpha}$ in a number of reconstitution experiments in isolated membranes (see refs. 12 and 13). Similarly, down-regulation of $G_{i\alpha}$, with no change in G_{β}, might also be expected to increase basal enzyme activity and to decrease the ability of inhibitory neurotransmitters to inhibit adenylate cyclase. Together, the two effects of glucocorticoids would appear to represent a concerted action of the hormone in up-regulating the adenylate cyclase system in brain. Indeed, exposure to glucocorticoids does increase the sensitivity of the cAMP generating system in a number of cultured cell lines (28–31), as well as in slices of rat cerebral cortex (10), and these actions are consistent with the present findings. However, glucocorticoids have also been reported to decrease neurotransmitter stimulation of cAMP production in brain slices depending on the brain region and neurotransmitter chosen for study (see refs. 4, 5, and 7–10). These different effects of glucocorticoids raise the possibility that the hormone has different actions on various G-protein subunits in different cell types and brain regions.

It is also possible that the glucocorticoid-induced changes in $G_{s\alpha}$ and $G_{i\alpha}$ serve to alter effector systems in addition to adenylate cyclase in brain. Thus, $G_{s\alpha}$ has been reported to mediate the effects of neurotransmitters on ion channels independent of its effects on adenylate cyclase, perhaps through the direct coupling of the receptors to the channels (32). Similarly, the different forms of $G_{i\alpha}$ have been implicated in the regulation of ion channels and phospholipases in addition to mediating neurotransmitter inhibition of adenylate cyclase (see ref. 14). Further characterization of glucocorticoid regulation of G_s and G_i could provide insight into other such effector systems.

Glucocorticoid regulation of G_s and G_i suggests that some of the complex behavioral and physiological effects of this steroid hormone on the brain are mediated at the level of G proteins. Important information regarding the precise molecular pathways through which this steroid hormone regulates brain function could, therefore, be obtained through further study of these phenomena. Alterations in $G_{s\alpha}$ and $G_{i\alpha}$ have been reported previously to be associated with a number of other pathological or pharmacological conditions in a variety of cell types. Thus, levels of $G_{s\alpha}$ have been shown to be altered in pseudohypoparathyroidism in humans (33), in human pituitary adenomas (34), in cultured GH_3 cells in response to dexamethasone (27), and in neuroblastoma × glioma cells in response to ethanol (23). Levels of $G_{i\alpha}$ have been shown to be altered in rat liver by experimentally induced diabetes (35) and in rat locus coeruleus under states of morphine tolerance and dependence (20). Alterations in these G proteins have been proposed to play a role in the altered physiological states of these various conditions. Taken together, the results of these studies provide further support for the view that alterations in G proteins represent a general mechanism by which neurons and nonneuronal cells adapt to chronic drug treatments and other chronic perturbations *in vivo*.

We thank Ms. Rose Terwilliger for excellent technical assistance. We also thank the following individuals for providing us with the valuable reagents that made this study possible: Drs. A. Gilman, J. Northup, A. Spiegel, and R. Reed. This work was performed while E.J.N. was a Pfizer Scholar. This work was also supported by the Alfred P. Sloan Foundation and by BRSG Grant RR05358 awarded by the Biomedical Research Support Grant Program, Division of Research Resources, National Institutes of Health (both to E.J.N.), and by the Abraham Ribicoff Research Facilities, Connecticut Mental Health Center, State of Connecticut Department of Mental Health.

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