Communication

\( \beta_2 \)-Adrenergic Receptors in Hamster Smooth Muscle Cells Are Transcriptionally Regulated by Glucocorticoids*  
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Steroid hormones modulate adrenergic receptor responsiveness and receptor number. To investigate the regulation of the \( \beta_2 \)-adrenergic receptor gene by glucocorticoids, we examined the effects of the synthetic glucocorticoid agonist triamcinolone acetonide on the expression of \( \beta_2 \)-adrenergic receptors in DDTMF-2 hamster smooth muscle cells. Glucocorticoid treatment (1 \( \times \) 10\(^{-7} \) M) produced a 2.2 \( \times \) 0.4-fold \((n = 8)\) increase in \( \beta_2 \)-adrenergic receptor number (maximum between 6 and 12 h) as determined by radioligand binding and a similar increase in catecholamine-stimulated adenylyl cyclase activity. Steady-state levels of \( \beta_2 \)-adrenergic receptor mRNA, analyzed by Northern blot hybridization, were increased 2.4 \( \times \) 0.4-fold \((n = 6)\) within 1 h, while actin mRNA levels were unchanged throughout the experiment. These steroid-induced increases in \( \beta_2 \)-adrenergic receptor mRNA returned to control levels by 24 h and were followed by a much slower decline in \( \beta_2 \)-adrenergic receptor in plasma membranes. The rate of \( \beta_2 \)-adrenergic receptor gene transcription, assessed by nuclear run-off transcription assays, increased 3.1 \( \pm \) 0.1-fold \((n = 2)\) in cells treated for 30 min with 1 \( \times \) 10\(^{-7} \) M triamcinolone acetonide. These studies indicate that glucocorticoids regulate the \( \beta_2 \)-adrenergic receptor-adenylate cyclase system by controlling the rate of transcription of the \( \beta_2 \)-adrenergic receptor gene and hence the responsiveness of the enzyme to catecholamine stimulation.

The \( \beta_2 \)-adrenergic receptor is a member of the family of membrane receptors involved in guanine-nucleotide regulatory protein (G-protein)-mediated signal transduction. One of the principal mechanisms for the regulation of these transmembrane signaling systems is the modulation of receptor number. This may be achieved in several ways. The loss of receptors which occurs following prolonged exposure to agonist is accompanied by a reduction in responsiveness. This phenomenon is termed "down-regulation" (1). Changes in receptor number may also arise as a consequence of pathophysiological conditions. For example, it has been known for some time that hyperthyroidism or conditions leading to elevated glucocorticosteroid levels can produce symptoms reminiscent of a hyperadrenergic state in the absence of detectable changes in serum catecholamine levels (2, 3). This form of regulation has been called "heterologous regulation," and it refers to the situation where hormones and drugs that are not specific ligands for adrenergic receptors can nevertheless regulate adrenergic receptor responsiveness.

Studies in vivo and in vitro have shown that glucocorticoids raise \( \beta_2 \)-adrenergic receptor levels and agonist-stimulated adenylyl cyclase activity (4--8). Since a major mode of steroid hormone action is the modulation of the rate of target gene transcription (9), we sought to determine whether the \( \beta_2 \)-adrenergic receptor is regulated by glucocorticoids at the level of gene expression.

**MATERIALS AND METHODS**

**Cell Culture**—DDTMF-2 cells (10) were grown in suspension culture in Dulbecco's modified Eagle's medium (GIBCO) supplemented with 20 mM Hapes, 1% fetal calf serum, 100 units/ml penicillin, and 100 \( \mu \)g/ml streptomycin. Cells were placed in fresh medium at 5 \( \times \) 10\(^5\) cells/ml and grown in suspension for 3 days, at which time cell density was 2.2 \( \pm \) 0.4 \( \times \) 10\(^6\) cells/ml (mean \( \pm \) S.E., \( n = 8 \)). The synthetic steroid triamcinolone acetonide (TA) was added to the culture from stock solutions prepared in 100% ethanol. The final concentration of ethanol in the medium did not exceed 0.1%. At the indicated times following the addition of drugs, aliquots of cells (1--5 \( \times \) 10\(^6\) for ligand binding; 1--2 \( \times \) 10\(^5\) for RNA) were harvested by centrifugation (800 \( \times \) g for 5 min). The cell pellets were flash-frozen in liquid nitrogen and stored at \(-80^\circ\)C until use. Cells grown in the presence of TA essentially cease dividing. Cell viability was estimated by trypan blue exclusion to be 85--90%.

**Preparation of Plasma Membranes and Assays for Radioligand Binding and Adenylyl Cyclase Activity**—Frozen cell pellets were thawed in buffer (75 mM Tris, pH 7.4, 12.5 mM MgCl\(_2\), 0.25 mM sucrose containing 5 \( \mu \)g/ml each of soybean trypsin inhibitor and leupeptin). Plasma membranes were prepared as previously described (11). Protein concentrations were determined by the method of Bradford (12). Binding of the \( \beta_2 \)-adrenergic receptor-specific ligand \([\_2^\text{H}]\text{cyanopindolol}\) \(([\_2^\text{H}]\text{CYP})\) was performed as previously described (13) in the absence or presence of 1 \( \mu \)M \((-\))-alpenadrol to define total and nonspecific binding, respectively. Catecholamine-sensitive adenylyl cyclase activity was measured in freshly prepared membranes as described previously (14, 15).

**Isolation of RNA**—Total cellular RNA was isolated by the cesium chloride gradient method of Chirgwin (16). Briefly, cells that had been flash-frozen at the time of harvest were thawed directly into 8 ml of 4 M guanidinium isothiocyanate, 2% sodium lauryl sarcosine, 50 mM Tris (pH 7.4), 10 mM EDTA, and 0.15 M 2-mercaptoethanol. The homogenate was passed through a 20-gauge needle three times to shear the DNA, layered over 2.0 ml of 5.7 M CsCl, 25 mM sodium acetate (pH 6), 1 mM EDTA, and centrifuged at 32,000 rpm in a Sorvall TH641 rotor for 20 h.

**RNA Blot Hybridization**—RNA was denatured in 1 M glyoxal, 50% (v/v) dimethyl sulfoxide, 10 mM sodium phosphate (pH 6.5) at 50 °C for 1 h and fractionated by electrophoresis through a 1.2% agarose gel as described (17). The RNA was transferred to Biodyne membranes (Pall Corp., Glen Cove, NY) and hybridized as described (18) to \( \beta_2 \)-adrenergic receptor (19) or actin (20) cDNA probes that were labeled by nick translation with \([\_3^\text{P}]\text{dCTP}\) (Du Pont-New England Nuclear) to specific activities of approximately \(3 \times 10^6\) to \(1 \times 10^7\) dpm/\(\mu\)g DNA. Following hybridization the filters were washed successively in 2 \(\times\) SSC (0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), 0.1% SDS at room temperature and 0.1 \(\times\) SSC, 0.1% SDS at 55 °C, and exposed to Kodak XAR or XRP film at \(-70^\circ\)C with two intensifying screens. Size estimates of discrete RNA species were established.

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\* The abbreviations used are: Hapes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; CYP, cyanopindolol; TA, triamcinolone acetonide; SDS, sodium dodecyl sulfate; kb, kilobase(s).
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lissed by comparison with an RNA Ladder and φX174 RF/HaeIII DNA fragments (Bethesda Research Laboratories) stained with methylene blue after blotting (21). Autoradiographic bands were quantitated by densitometric scanning.

Nuclear Run-off Transcription Assay—Following the addition of either 1 × 10^{-7} M TA or ethanol vehicle, DDT;MF-2 cells were harvested by centrifugation and washed in a buffer containing 10 mM Tris, pH 7.5, 10 mM KCl, 3 mM MgCl₂, 3 mM dithiothreitol. Nuclei were prepared exactly as described (22) with the following exceptions. The nuclei were sedimented (25,000 rpm in a Sorvall TH641 rotor at 4°C) through a 3.0-ml cushion of 2.0 mM methylene blue after blotting (21). Autoradiographic bands were quantitated by densitometric scanning. The filters were exposed to Kodak XRP film. Autoradiographs were quantitated by densitometric scanning, and the increase in β_2-adrenergic receptor mRNA (includes major and minor hybridizing species) relative to the untreated control by 10 h. Throughout the experimental period there was no appreciable change in actin mRNA levels (Fig. 2B). Therefore, actin mRNA was used as an internal control for minor fluctuations in total RNA applied to the gel. When exposed relative to actin, there was an overall doubling of β_2-adrenergic receptor mRNA in the first hour, which decayed to less than 0.5-fold at later time points (Fig. 2C). The average maximal increase in β_2-adrenergic receptor mRNA from several experiments was 2.4 ± 0.4-fold (n = 6).

We next utilized nuclear run-off transcription assays to determine whether this doubling of steady-state β_2-adrenergic receptor mRNA levels was due to stimulation of the rate of β_2-adrenergic receptor gene transcription. In cells treated for 30 min with 1 × 10^{-7} M TA there was a 3-fold increase in

Results

Preliminary dose-response experiments indicated that maximal effects on the parameters measured in these studies were observed with 1 × 10^{-7} M TA; therefore, this concentration of the hormone was used throughout these investigations. Following the addition of 1 × 10^{-7} M TA to DDT;MF-2 cells, the β_2-adrenergic receptor number increased 2-fold. Fig. 1 is representative of eight independent experiments in which the β_2-adrenergic receptor number steadily increased to a maximum of 2.6-fold at 8 h. While there was some variation between individual experiments, the peak of induction (2.2 ± 0.4-fold) typically appeared between 6 and 12 h, plateaued, and then gradually declined over the next 2 days. In untreated control cells receptor number remained constant during the 48-h period (data not shown). From saturation binding studies there was no apparent change in the dissociation constant for [125I]CYP between control cells and treated cells (data not shown), in agreement with previous reports (5, 6, 8). In cells treated with 1 × 10^{-7} M TA for 12 h and 20 h, isoproterenol-stimulated adenylate cyclase activity was elevated 38 and 56%, respectively, over control cells (data not shown). Fluo-

![Fig. 1. Binding of [125I]cyanopindolol to DDT, MF-2 membranes following glucocorticoid treatment.](image)

![Fig. 2. Effect of glucocorticoid treatment on β_2-adrenergic receptor (β_2-AR) and actin mRNA levels in DDT;MF-2 cells.](image)
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FIG. 3. Transcriptional activation of the β2-adrenergic receptor gene by glucocorticoid. DDT,MF-2 cells grown in the absence (−) or presence (+) of 1 × 10−5 M TA for 30 min were harvested for isolation of nuclei. Transcript elongation in isolated nuclei was allowed to proceed in the presence of [α-32P]UTP, and the 32P-labeled run-off transcripts were hybridized to plasmids (2 μg/slot) bound to nitrocellulose. pMB1.3 contains the 1.3-kb HindIII fragment of the hamster β2-adrenergic receptor cDNA (19). pGEM is a control vector containing no insert. Autoradiograms were scanned densitometrically to determine the average increase in β2-adrenergic receptor transcription rate, which was 3.10 ± 0.08-fold (mean ± S.D., n = 2).

nascent β2-adrenergic receptor transcripts (Fig. 3). Hybridization to the hamster β2-adrenergic receptor cDNA insert in plasmid pMB1.3 (19) was specific as indicated by the lack of hybridization to the control plasmid, pGEM.

Discussion

These results demonstrate that the β2-adrenergic receptor in DDT,MF-2 cells is regulated by glucocorticoids. The increase in receptor density and isoproterenol-stimulated adenylate cyclase activity are essentially identical to results which have been described previously in other tissues and species (4–8). Our experiments extend these previous findings by documenting for the first time the role of regulation of β2-adrenergic receptor gene expression in this process.

Immediately following the addition of steroid to the cells there was a rapid rise in steady-state levels of β2-adrenergic receptor mRNA, which preceded the appearance of new receptors in the membrane. The peak in accumulation of β2-adrenergic receptor mRNA was reached within 1–2 h. Thus, the doubling of receptors can be attributed to equivalent increases in steady-state β2-adrenergic receptor mRNA levels. These elevated levels of β2-adrenergic receptor mRNA were not maintained, however, and they quickly returned to the level of the untreated control. Similar findings of transient mRNA accumulation and transcriptional enhancement have been reported for other steroid-regulated genes. For example, Granner and colleagues (25) have observed that expression of the rat phosphoenolpyruvate carboxykinase gene is stimulated 8-fold by glucocorticoids within 1 h and then subsequently declines to 2-fold and stabilizes at that lower level. Likewise, dexamethasone has been shown to produce a several-fold increase in angiotensinogen mRNA, but this induction was temporary, approaching control levels by 24 h (26). The mechanisms responsible for this type of regulation are currently unknown but may be related to the ability of glucocorticoids to down-regulate their own receptors (27, 28), a process recently reported (29) to involve decreased transcription of the glucocorticoid receptor gene.

The return of β2-adrenergic receptor mRNA levels to control values after approximately 1 day was followed by a more gradual decline of the β2-adrenergic receptor number in the membrane. In other experiments we have found that by approximately 4 days, β2-adrenergic receptor levels had also returned to control (data not shown). The half-life of the β2-adrenergic receptor has been estimated to be ~30 hours (30).

At this rate, after 90 h only 10–15% of the steroid-induced receptors would be estimated to remain, which agrees well with our observations. The decrease in β2-adrenergic receptor mRNA below the level of the uninduced control may be more apparent than real. Our culture conditions, which include 5% fetal calf serum, probably result in some modest level of glucocorticoid stimulation in the untreated control, as has been observed in other systems (31). In cells exposed to a serum-free formulation for 2 days we have observed a significantly lower basal level of β2-adrenergic receptor and consequently a higher level of β2-adrenergic receptor induction (5-fold; n = 2) following hormone treatment (data not shown). Thus, the true “basal” level of β2-adrenergic receptor mRNA to which the system returns may be lower than that observed in “control” cells prior to the addition of the steroid. However, these conditions were not favorable for cell viability and growth; therefore, we conducted all of our experiments in serum-containing medium.

For most steroid-responsive genes studied, expression is largely controlled by changes in the rate of transcription, although significant differences in message stability have also been described (9). By nuclear run-off transcription assays we detected a 3-fold increase in the rate of transcription of the β2-adrenergic receptor gene in cells treated for 30 min with TA. Therefore, these results indicate that the increase in steady-state β2-adrenergic receptor mRNA levels is due to enhanced transcription of the β2-adrenergic receptor gene.

Heterologous hormonal regulation of β2-adrenergic receptor, particularly by steroid hormones, has been the subject of considerable investigation (2–8), and it has been speculated that control of β2-adrenergic receptor gene transcription may be the underlying mechanism. This notion was further supported by the discovery of several consensus glucocorticoid response elements in the cloned β2-adrenergic receptor gene (32, 33). While we do not know to which, if any of these glucocorticoid response elements, functional significance can be ascribed, this is an obvious area for further study.

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