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Steroids induce acetylcholine receptors on cultured human muscle: Implications for myasthenia gravis

(autoantibodies/dexamethasone)

IRVING KAPLAN*, BRUCE T. BLAKELY, GRACE K. PAVLATH, MARILYN TRAVIS, AND HELEN M. BLAU†

Department of Pharmacology, Stanford University School of Medicine, Stanford, CA 94305-5332

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ABSTRACT **Antibodies to the acetylcholine receptor (AChR), which are diagnostic of the human autoimmune disease myasthenia gravis, block AChR function and increase the rate of AChR degradation leading to impaired neuromuscular transmission. Steroids are frequently used to alleviate symptoms of muscle fatigue and weakness in patients with myasthenia gravis because of their well-documented immunosuppressive effects. We show here that the steroid dexamethasone significantly increases total surface AChRs on cultured human muscle exposed to myasthenia gravis sera. Our results suggest that the clinical improvement observed in myasthenic patients treated with steroids is due not only to an effect on the immune system but also to a direct effect on muscle. We propose that the identification and development of pharmacologic agents that augment receptors and other proteins that are reduced by human genetic or autoimmune disease will have broad therapeutic applications.**

Myasthenia gravis (MG) is a human autoimmune disorder that affects neuromuscular function by impairing synaptic transmission. MG patients have circulating autoantibodies directed against nicotinic acetylcholine receptors (AChRs) concentrated on skeletal muscle at neuromuscular junctions (1–3). These antibodies reduce the sensitivity of muscle to acetylcholine by blocking ligand binding sites and by increasing the rate of AChR degradation (4–10). Glucocorticoids used in conjunction with acetylcholinesterase (AChE) inhibitors are currently among the most effective pharmacologic treatments of the debilitating clinical symptoms of MG and may be responsible for the 50% reduction in mortality in the past two decades (11). The recovery of motor strength after glucocorticoid therapy is thought to result from the well-documented immunosuppressive effects of steroids (12, 13). However, the titer of anti-AChR antibodies in MG patients treated with steroids often does not correlate with the severity of the disease or incidence of death (11, 14). We demonstrate here that the glucocorticoid dexamethasone (Dex) directly affects cultured human myotubes by inducing AChR synthesis and increasing total surface AChRs 2-fold. In contrast, Dex has no effect on other parameters of muscle differentiation such as fusion index, AChE activity, and sarcomeric actin transcript accumulation. The effect of Dex on AChR levels is evident in the presence of sera from MG patients, indicating that the therapeutic benefits of corticosteroid therapy are likely to derive from effects not only on the immune system but also on muscle tissue. These results suggest that therapeutic strategies directed at promoting tissue-specific gene expression will prove useful in MG as well as other human genetic and autoimmune disorders characterized by reduced levels of proteins including cell surface receptors.

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MATERIALS AND METHODS

Muscle Cultures. Individual muscle clones were isolated as described (15) from the vastus lateralis muscle of three individuals (XXVI, XXXII, and XXVIII aged 2, 7, and 63 years, respectively), stored frozen, thawed, and used in pools of three. Muscle samples were obtained coincident with surgical treatment for orthopedic nonmuscle problems and at autopsy in accordance with the guidelines of the Human Subjects Committee of Stanford University. Cultures were incubated at 37°C in 5% CO_2/95% air at 100% humidity. Myoblasts were induced to proliferate in growth medium (GM) containing Ham's F10 medium with 0.5% chicken embryo extract and 15% (vol/vol) fetal calf serum. To induce fusion, GM was replaced with a serum-free medium containing Dulbecco's modified Eagle's medium (DMEM) with Dex (Sigma) overnight, followed by a fusion medium (FM) containing DMEM with 15% (vol/vol) horse serum and Dex. Unless otherwise indicated, Dex was used at 25 nM. The fusion medium was renewed every 4 days. All media contained penicillin G (200 units/ml) and streptomycin sulfate (200 μg/ml) and were obtained from GIBCO, except as indicated. In experiments examining the effects of normal or myasthenic serum on the metabolism of human AChRs, horse serum was replaced for the indicated period with human serum that had been heated to 56°C for 20 min to inactivate complement.

Normal and MG Sera. MG sera were the generous gift of J. Lindstrom (Salk Institute, La Jolla, CA) and L. Steinman (Stanford University, Stanford, CA). Normal sera were obtained from individuals with no known neuromuscular or autoimmune disease in accordance with the guidelines of the Human Subjects Committee of Stanford University.

AChR Accumulation on Cell Surface. To assay total surface AChRs, parallel sets of cultures were saturated with ^{125}I-labeled α-bungarotoxin (^{125}I-α-BuTx) at 25 nM (122–147 μCi/mM; 1 Ci = 37 GBq; NEN) for 30 min at 37°C, in DMEM supplemented with 2% horse serum (Kansas City Biologicals) and bovine serum albumin (1 mg/ml; Sigma), as described (15). Unbound toxin was removed by washing four times for a total of 60 min at 37°C with the above medium containing unlabeled α-BuTx (0.1 μM, Sigma). The ^{125}I-α-BuTx-labeled AChRs were then extracted by lysis with 0.1 M NaOH and collected with two rapid washes of isotonic phosphate-buffered saline (PBS), and then radioactivity was measured in a Beckman 5500 γ counter. All AChR assays were performed in duplicate or triplicate. Each value was corrected for nonspecific binding determined by exposing replicate

Abbreviations: Dex, dexamethasone; AChR, acetylcholine receptor; MG, myasthenia gravis; α-BuTx, α-bungarotoxin; ^{125}I-α-BuTx, ^{125}I-labeled α-BuTx; AChE, acetylcholinesterase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

*Present address; Department of Radiation Therapy, Stanford University School of Medicine, Stanford, CA 94305-5332.
†To whom reprint requests should be addressed.

cultures to excess unlabeled α-BuTx (Boehringer Mannheim) at 1 μM prior to incubation with ^{125}I-α-BuTx (15).

AChR Synthesis and Insertion. The rate of *de novo* AChR synthesis and insertion was determined as the rate of appearance on the cell surface of AChRs capable of binding labeled toxin. At various time intervals after exposure to unlabeled α-BuTx (1 μM for 20 min), cultures were saturated with ^{125}I-α-BuTx (25 nM for 30 min).

AChR Degradation. Degradation of AChR was assayed as the release into the culture medium of ^{125}I-labeled tyrosine derived from degraded ^{125}I-α-BuTx–AChR complexes, as described (15). Parallel sets of cultures were first saturated with ^{125}I-α-BuTx for 30 min at 37°C. The unbound toxin was then removed by four washes with culture medium at 37°C. At various times, samples of cells and medium were separately collected and the fractional amount of degradation was calculated as follows: (cpm bound to cells)/(cpm bound to cells + cpm found in medium as labeled tyrosine by P2 column chromatography). In agreement with other species (9, 16), in the presence or absence of antibodies to AChR, human AChR degradation consistently follows first-order kinetics.

An alternate method for assaying AChR degradation indirectly as the release of radioactivity into culture medium at various times after exposure of muscle cultures to ^{125}I-α-BuTx has been widely used. However, the release of undegraded ^{125}I-α-BuTx into the medium can complicate this assay, especially when the levels of AChR are relatively low (9, 15). Thus, the rate of AChR turnover is more accurately determined by assaying the rate of appearance in the media of ^{125}I-labeled tyrosine, a direct by-product of the degradation of toxin–receptor complexes (9, 15, 16).

AChE Enzyme Activity. Muscle cultures were rinsed twice with PBS at 4°C, and cells were removed by scraping dishes, homogenized in buffer A (17), and centrifuged. Supernatants were assayed by spectrophotometer for AChE activity according to the method of Ellman (18) by using 0.33 mM acetylthiocholine iodide as the substrate. The hydrolysis of acetylthiocholine iodide was inhibited by 99% in parallel assays by treatment with eserine, a selective inhibitor of AChE. AChE activity was corrected for spontaneous hydrolysis of the substrate alone and for nonspecific activity in cell extracts in the absence of substrate. The AChE enzyme assays were linear with respect to both time and enzyme concentration. Protein concentration was determined by a modification of the method of Lowry, as described (15), and AChE activity was expressed as units per mg of protein.

Northern Blot Analysis. Total RNA was isolated by the method of Chirgwin (19), electrophoresed on 1% agarose/

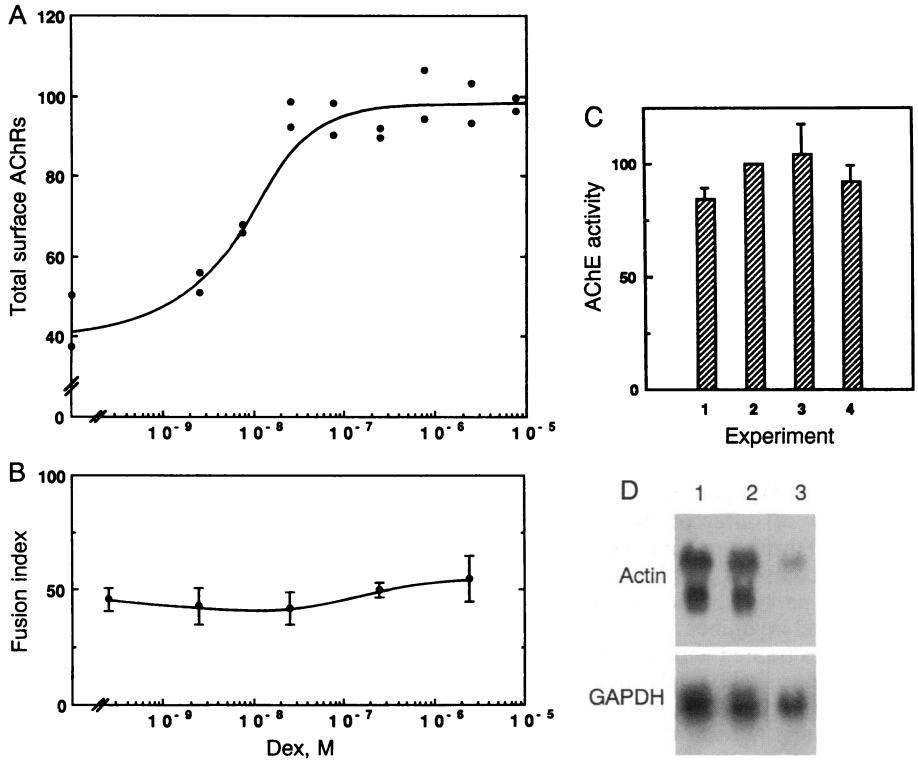

FIG. 1. (*A*) Levels of total surface AChRs on cultured human muscle increase in response to Dex. A dose–response curve was generated by exposing human myoblasts to Dex during a 6-day period of differentiation. At the end of this period, the number of AChR sites on the muscle cell surface was determined by saturation with ^{125}I-α-BuTx, as described (15). Total surface AChRs are expressed as fmol of ^{125}I-α-BuTx bound per mg of protein. (*B*) Fusion index is not altered by exposure to different concentrations of Dex. The fusion index, expressed as percent of total nuclei in differentiated multinucleated myotubes, was determined for cultures fixed in 100% methanol and stained with hematoxylin and eosin. For each concentration of Dex, four to six random fields containing an average of 3000 nuclei were scored in duplicate dishes and expressed as the mean ± SD. The results in *A* and *B* are representative of three dose–response experiments that used cultured muscle cells isolated from three individuals, XXVI, XXXII, and XXVIII. (*C*) AChE activity is not altered by exposure to Dex. Cultures from two individuals, XXVI (bars 1 and 2) and XXVIII (bars 3 and 4) were grown with Dex or without Dex (control) and assayed on day 6 (bars 1–3) or day 8 (bar 4) for AChE activity. The data were corrected for nonspecific activity and expressed as percent control. Data (mean ± SEM) are from duplicate assays in duplicate dishes. In accordance with previous findings (27), Dex resulted in increased proliferation and cell density as determined by mg of protein per dish, with a proportionate increase in muscle-specific AChE. After 8 days, cells without Dex came off the dish, whereas cells with Dex exhibited adherence and persisted for longer periods of time. (*D*) Accumulation of sarcomeric actin transcripts is not increased by Dex. For Northern blot analysis of total RNA (10 μg) isolated from human muscle cells (sample XXXII) cultured in the presence (lane 1) or absence (lane 2) of Dex for 5 days, RNA was hybridized sequentially to probes for sarcomeric α cardiac and skeletal actins (lower actin band) and ubiquitous β and γ actins (upper actin band) and for GAPDH, which serves as a marker for comparing mRNA levels. Sample XXVIII gave similar results. RNA from the human hepatoma line HepG2 (28) served as a negative control for muscle-specific transcripts (lane 3).

6.7% formaldehyde gels, blotted, and UV-crosslinked onto nylon membrane (Nytran) according to the manufacturer (Schleicher & Schuell). Prehybridizations and hybridizations were performed in 50% (vol/vol) formamide/5× SSPE/5× Denhardt's solution/1% SDS/sheared calf thymus DNA (200 μg/ml)/yeast tRNA (200 μg/ml)/poly(A) (50 μg/ml) at 42°C (1× SSPE = 0.18 M NaCl/10 mM sodium phosphate, pH 7.4/1 mM EDTA; 1× Denhardt's solution = 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin). After a 24-hr hybridization, blots were washed for three 20-min periods in 2× SSPE/1% SDS at 55°C and then exposed to film with an intensifying screen. Between hybridizations, probes were removed by washing blots in 50% formamide/5× SSPE at 65°C for 1 hr.

Probes were prepared by random-hexamer extension (20) using a kit (Amersham) and were labeled to a specific activity of $>10^8$ cpm/μg. The cDNA probes used were a 500-base-pair human actin coding region fragment originally from pHMαA-PX (21) subcloned into pGEM-Blue (Promega) and provided by Lydia Pan (Stanford University, Stanford, CA), and a 1.2-kilobase rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (22). The actin film shown was exposed for 12 hr and the GAPDH film was exposed for 24 hr.

RESULTS

Effect of Dex on AChR Metabolism. Myoblasts were isolated from human muscles, cloned to eliminate nonmuscle cells, cultured, and induced to fuse into striated, contracting, multinucleated myotubes (15). The myotubes synthesized a number of muscle-specific products (23, 24), including the AChR (15). Human AChR has ligand-binding properties and rates of synthesis and degradation similar to the AChR of muscle cultures from other species (15, 16, 25, 26). Dex induces human cell surface AChRs to levels 2-fold above the level of controls. The dose–response curve in Fig. 1*A* shows that a continuous exposure to Dex leads to an increased accumulation of total surface AChRs assayed by binding of ^{125}I-α-BuTx, a ligand with a high affinity and specificity for the nicotinic AChR. The observed half-maximal response at 10 nM is typical of other Dex-inducible effects in cultured cells (29–33).

To rule out the possibility that Dex acts nonspecifically by enhancing the overall extent of differentiation of human muscle cultures, we performed three types of experiments. (*i*) We examined whether the increase in AChR was due to an increase in the fusion index or percent of total myoblasts that had fused to form differentiated myotubes. As shown in Fig. 1*B*, the fusion index in cultures treated with various concentrations of Dex did not differ significantly from that in untreated controls. Thus, the hormone increased the accumulation of AChRs per myotube but not the proportion of differentiated myotubes per dish. (*ii*) We examined the effects of Dex on AChE activity. This was particularly important since an increase in AChE could conceivably negate the effects of increased AChRs at the neuromuscular junction. Duplicate cultures of human muscle cells from two individuals were differentiated in either the presence or absence of Dex and assayed for AChE activity in two series of experiments. The results shown in Fig. 1*C* indicate no significant increase in AChE activity in Dex-treated relative to untreated (control) cultures. (*iii*) We examined whether Dex altered the levels of muscle-specific transcripts encoding sarcomeric actins in human muscle cultures. The levels of the sarcomeric transcripts in different samples can be directly compared, since GAPDH serves as a control for mRNA levels in diverse cell types (34). As shown in Fig. 1*D*, Dex does not significantly alter the accumulation of actin transcripts. Moreover, no difference between treated and untreated cultures in maturation was detected when fetal, neonatal, and adult myosin heavy chain isozymes were assayed (data not shown). In agreement with the findings of others that Dex does not alter creatine kinase activity (27, 35), we conclude that the effect of Dex on AChR accumulation is relatively specific (35) and not the result of a general enhancement of muscle differentiation in culture.

Table 1. Dex enhances AChR synthesis in human muscle cultures

Dex	AChR synthesis and insertion, fmol per mg per hr	AChR degradation, % per hr	Total surface AChR, fmol per mg of protein
−	4.5 ± 0.4	3.5 ± 0.5	41.1 ± 8.1
+	9.1 ± 1.2	3.9 ± 1.1	84.5 ± 3.8

Rate of AChR synthesis and insertion was defined as the rate of appearance of AChR on the plasma membrane capable of binding labeled toxin. At 4.0- and 4.5-hr intervals after exposure to unlabeled BuTx (1 μM for 20 min), cultures in four experiments were saturated with ^{125}I-α-BuTx. As shown, this rate was constant for 6 hr (15). Rate of AChR degradation was determined by assaying the rate of appearance in the medium of ^{125}I-labeled tyrosine, the degradation product of ^{125}I-α-BuTx-AChR complexes, by P2 column chromatography at 13.5 and 20.0 hr in five experiments. The fractional amount of degradation was calculated at these time intervals and the percent of surface AChR degraded per hr was determined. This rate follows first-order kinetics (9, 15, 16) and parallels the loss of receptor sites. Total surface AChR sites were determined at the onset of each of four experiments as fmol of ^{125}I-α-BuTx bound per mg of protein by saturating AChRs with ^{125}I-α-BuTx at 20 nM for 30 min at 37°C.

The increase in AChR on cultured human muscle upon exposure to Dex was due to an increase in the rate of AChR synthesis. At any given time, the number of AChRs on the cell surface is the net result of AChR synthesis and degradation. As shown in Table 1, the 2-fold increase in surface AChRs induced by Dex could be attributed almost entirely to an increase in the rate of *de novo* AChR synthesis, determined by measuring the ^{125}I-α-BuTx binding at various times after saturation with unlabeled α-BuTx (15, 16). On the other hand, degradation, assayed as the rate of accumulation in the medium of ^{125}I-labeled tyrosine, the metabolic product of ^{125}I-α-BuTx–AChR complexes, followed first-order kinetics and did not differ appreciably in parallel cultures that differ-

Table 2. Sera from MG patients accelerate the degradation of human muscle AChR

Control	AChR half-life, hr	Myasthenic patient	AChR half-life, hr
N1	8.7 ± 1.5	MG1	4.4 ± 0.2
N2	11.0 ± 1.6	MG2	4.8 ± 0.4
N3	9.9 ± 0.6	MG3	4.6 ± 0.3

Control sera were from the following three healthy individuals with no known autoimmune disease: N1 (I.K.), N2 (S.A.), and N3 (J.K.). Myasthenic sera were from the following three individuals: MG1, moderate generalized, anti-AChR antibody titer 225 nM; (MG2) mild generalized, anti-AChR antibody titer 13 nM; and MG3, clinical status unknown. Antibody titers against human AChR were determined by J. Lindstrom by radioimmunoassay (36). Data are from three replicate dishes (mean ± SEM). AChR sites on differentiated human muscle cultures on day 6 in fusion medium containing Dex were first saturated with ^{125}I-α-BuTx and then exposed to 15% normal human serum or 15% myasthenic serum for 8 hr. The accumulation in the medium of ^{125}I-labeled tyrosine, the specific degradation product of ^{125}I-α-BuTx, was monitored by P2 column chromatography and expressed as the percent of initial ^{125}I-α-BuTx binding sites destroyed per hour and the half-life was calculated as described (15). The degradation of human AChR (15), like that of other species (9, 16), consistently follows first-order kinetics. Data are from three replicate dishes (mean ± SEM). The differences in rates of degradation for control and myasthenic sera are significant by the Student's *t* test ($P < 0.001$).

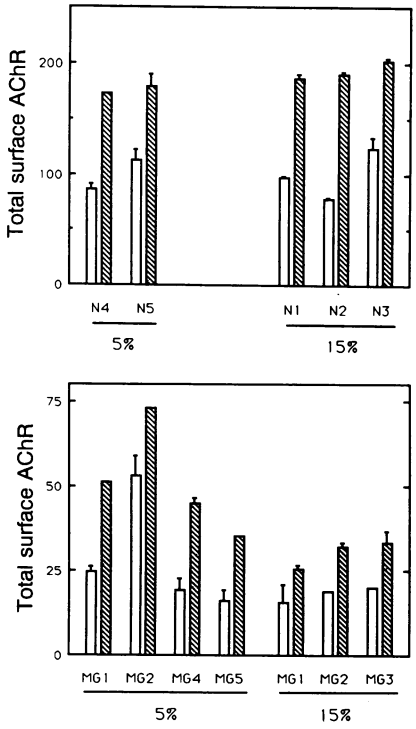

FIG. 2. Dex increases the levels of total surface AChR binding sites in the presence of MG sera. Replicate cultures of human muscle cells in two series of experiments were induced to differentiate for 5 days with or without Dex (50 nM) and then exposed for 16 hr to either 5% or 15% sera from controls or from myasthenic individuals, as indicated. The dishes were then assayed for total surface AChR. The ^{125}I-α-BuTx bound per mg of protein for cultures differentiated in the absence (open bars) and presence (hatched bars) of Dex is shown as the mean ± SEM for each test serum. The data are expressed as percent of control, calculated as the average ^{125}I-α-BuTx binding sites without Dex for five healthy controls: N4 (B.A.) and N5 (B.B.) in the 5% serum experiments and N1 (I.K.), N2 (S.A.), and N3 (J.K.) in the 15% serum experiments, respectively. Normalization in this manner is appropriate since, as noted (9, 15), the absolute amount of AChR per mg of protein is similar for parallel cultures within an experiment but differs among experiments due to differences in myogenic clones and culture conditions. Myasthenic sera were from patients with MG as follows: MG1, moderate generalized, anti-AChR antibody titer 225 nM; MG2, mild generalized, anti-AChR antibody titer 13 nM; MG3, unknown; MG4, chronic severe, anti-AChR antibody titer 38 nM; MG5, mild generalized, anti-AChR antibody titer 82 nM. Horse serum (10%) was added in the 5% sera conditions to maintain the serum concentration at 15%.

entiated in the presence or absence of Dex. Although the absolute values for degradation in Tables 1 and 2 cannot be directly compared due to the well-documented variation among experiments, the relative values within an experiment are comparable (9, 15). Thus, the rate of insertion of newly synthesized AChRs in the membrane increased with Dex, but the rate of degradation or half-life of AChR sites remained constant.

Effect of MG Serum on AChR Expression. Sera from three MG patients with different antibody titers and clinical courses accelerated degradation of human AChRs (Table 2). In human muscle cultures exposed to myasthenic sera, surface AChRs had a half-life of 4.6 ± 0.2 hr. By contrast, in parallel cultures incubated with sera from three normal individuals, the average half-life of surface AChRs was 9.9 ± 1.1 hr. The nearly 2-fold acceleration of degradation of the human AChR in the presence of MG antisera is significant ($P < 0.001$) and is similar to the kinetics of AChR degradation observed in muscle culture systems of other species exposed to human myasthenic sera (37).

The increase in AChR synthesis observed in cultured myotubes treated with Dex was found to compensate, in part, for the decrease in ^{125}I-α-BuTx binding observed in cultures exposed to MG sera (Fig. 2). When replicate dishes of human myotubes were incubated for 16 hr in medium containing 5% or 15% (vol/vol) myasthenic sera from various patients, the total surface AChRs assayed by saturating binding sites with ^{125}I-α-BuTx decreased. On myotubes cultured in the presence of Dex, however, the number of AChR binding sites was nearly 2-fold greater. With the exception of the MG2 serum, which had the lowest antibody titer, no significant differences between the low and high concentrations of MG sera were observed, presumably because the concentrations of IgG in both cases were relatively high.

DISCUSSION

The experiments presented here underscore the advantage of using human muscle cultures to study human neuromuscular disorders. The response to Dex may be species-specific, since we have not observed a similar increase in AChR synthesis in the mouse muscle cell line C2C12 (I.K. and H.M.B., unpublished observation). A species difference in response to Dex has also been reported for cultured bone cells (38). Moreover, AChRs of different species differ structurally; erabutoxin-b, a neurotoxin in the venom of a Japanese sea snake, binds to rodent but not human AChR at neuromuscular junctions (39). In addition, radioimmunoassays reveal that MG titers for rat and mouse AChR are at most 15% those obtained from human AChR (40). That only a minor proportion of the spectrum of antibodies present in MG sera binds to rodent AChR can be misleading, not only in assessing antibody titers, but also in the interpretation of assays examining the effects of MG antibodies on AChR function or degradation. Thus, species differences may account for disparate results in studies examining whether disease severity is correlated with antigenic modulation in culture (9, 41).

Dex stimulates the expression of differentiation-specific properties in several cell types in addition to muscle. The half-maximal dose, lag time, and fold induction seen with Dex in tissue culture are similar for human muscle AChR, adipogenic differentiation of 10T½ and 3T3 cells (31, 33), expression of several enzymes typical of differentiating fetal lung (29, 30), stimulation of glycogen storage in hepatocytes (42), and collagen synthesis by vascular smooth muscle cells (32). For lung, the induction of differentiation observed *in vitro* has had useful application *in vivo* in the prevention of respiratory distress syndrome (43, 44). Moreover, the effective therapeutic doses of Dex (5–20 mg/day) are in the range of those used in the treatment of MG (45). Thus, effects on properties of differentiation observed in human cells in tissue culture can be related to the metabolism of these cells in the individual.

The exact mechanism by which Dex increases the expression of surface AChR remains unknown. Like other cell types that differentiate in response to Dex (29–33), the increase in the levels of total surface AChR in muscle cells requires at least 24 hr to develop and is not reversed upon withdrawal of the hormone but persists for up to 6 days (I.K. and H.M.B., unpublished observation). This prolonged effect suggests that Dex acts by inducing *de novo* AChR synthesis rather than by stimulating insertion of preexisting AChR into the plasma membrane, although a post-translational effect on glycosylation or phosphorylation cannot be ruled out (46). As we (15) showed, when synthesis is inhibited, the intracellular AChR pool is depleted and appears on the cell surface within 6 hr.

Therapeutic strategies have primarily been directed at increasing the function of the reduced number of AChR at the

neuromuscular junctions of MG patients. AChE inhibitors are effective in potentiating the effect of acetylcholine at synapses by inhibiting its degradation. During the past two decades, steroids have often been used in conjunction with AChE inhibitors. Significant clinical improvement occurs in 90% of patients treated with steroids, and the need for anticholinesterase drugs in these patients is usually decreased (45). The efficacy of steroids may, in part, be due to their immunosuppressive effects, since a reduction in anti-AChR antibody titer is often noted (10). However, antibody titer remains poorly correlated with disease severity, clinical course, and mortality (9, 14). The data presented here suggest another mechanism by which glucocorticoids act to ameliorate the symptoms and progression of MG. In addition to having immunosuppressive effects, steroids may have a direct effect on human muscle AChR accumulation, leading to increased AChR function and improved synaptic transmission. The observed reduction in ^{125}I-α-BuTx binding in cultures treated with MG sera could result from an increase in AChR degradation or the blocking of ligand-binding sites caused by anti-AChR antibodies (4–10). Either of these mechanisms leads to reduced physiological activity of the receptors. Our results demonstrate that the loss of activity can be counteracted in part by steroids.

Several studies indicate that the therapeutic efficacy of pharmacologic agents may derive from their effects in augmenting the synthesis of proteins, including receptors. Steroids used in conjunction with calcitonin lead to marked clinical improvement in patients with hypercalcemia of malignancy (47). The basis for the increased efficacy of calcitonin in the presence of glucocorticoids may well be due to increased synthesis of calcitonin receptors, which are down-regulated in response to continuous exposure to calcitonin (48). Similarly, the beneficial effect of steroids seen in patients with the human genetic disease Duchenne's muscular dystrophy may be due to increased synthesis of the protein dystrophin, as discussed by Brown (49). Glucocorticoids are known to enhance gene expression in many tissues at a transcriptional and post-transcriptional level (for review, see ref. 50). Another inducer, γ-interferon, leads to increased levels of cytochrome *b*, resulting in a partial correction of the phagocyte defect in superoxide production in patients with chronic granulomatous disease (51). Thus, with our results, these data suggest that therapeutic strategies directed at increasing tissue-specific gene expression will prove useful in a number of autoimmune and genetic diseases characterized by reduced levels of proteins including cell surface receptors.

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