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Title: INTERACTION BETWEEN GLUCOCORTICOID STATUS AND β-ADRENERGIC AGONISTS IN CONTROL OF RAT MUSCLE GROWTH AND PROTEIN DEGRADATION

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The objectives of this study were to examine glucocorticoid-dependency for acute anabolic actions of cimaterol and mechanisms by which this is mediated. Forty eight male Sprague Dawley rats (265 - 296 g) were assigned to 6 treatments: control (sham-adrenalectomized; Sham), Sham-plus dietary cimaterol (25 ppm), adrenalectomized (Adx), Adx plus cimaterol, Adx plus daily injections of dexamethasone (Dex, 1.2 μg/100 g BW/day; AdxD) and AdxD plus cimaterol (AdxDC). Animals not receiving Dex received vehicle. Animals were maintained for eight days in individual cages with water containing 0.9 % NaCl and feed (AIN-76 diet) available ad libitum. Urine was collected on day 1, 2, 4 and 7 for assessment of N^{τ}-methylhistidine (NMH) excretion. Adrenalectomy reduced weight gain ($p < .05$) and Dex treatment further reduced weight gain ($p < .05$). Cimaterol stimulated weight gain in AdxD animals ($p < .05$) but not in

Sham and Adx animals. Similar effects of Dex and cimaterol status on muscle weights were also observed. These observations, however, did not confirm the glucocorticoid-dependency for acute anabolic actions of cimaterol, because AdxDC animals did not gain faster than AdxC animals. Instead, we proposed that ability of cimaterol to enhance growth and muscle weight in Sham and AdxD animals may relate to its ability to antagonize the growth-inhibiting actions of glucocorticoids. Protein synthesis of isolated epitrochlearis muscle was only affected by adrenalectomy in cimaterol-fed animals. In contrast, urinary NMH excretion of Adx and AdxD animals was increased by several-fold, compared to sham-controls. This implies that reduced muscle weight caused by Adx and Dex is mediated by accelerated myofibrillar protein degradation. Dex treatments increased ($p < .05$) urinary NMH excretion on Day 2 but reduced ($p < .05$) it on Day 7, compared to their Adx counterparts suggesting a temporal control of muscle protein degradation by dexamethasone. Several proteinases were examined in this study. These included cathepsins B, D and L, neutral proteinase and calcium-dependent proteinases(CDPs). Cathepsin L activity was slightly increased ($p < .05$) by Adx and reduced ($p < .05$) by Dex treatment. Neutral proteinase activity was increased ($p < .05$) by Adx but was not affected by Dex treatment. Cathepsin D activity was opposite the response of cathepsin L. Activities of both mCDP and μCDP were reduced ($p < .05$) by Adx, and Dex treatment did not further reduce activities of mCDP or μCDP. Cathepsin activity was not affected by glucocorticoid status. Neutral proteinase was reduced ($p < .05$) by cimaterol irrespective of glucocorticoid status. Cathepsin B response to cimaterol was opposite to the direction of neutral proteinase. Cathepsin L was increased ($p < .05$) by cimaterol in Sham and AdxD rats. Cathepsin D activity was increased

($p < .05$) in Adx rats and was reduced ($p < .05$) in AdxD rats by cimaterol. Both mCDP and μCDP activities were reduced ($p < .05$) in Sham and increased ($p < .05$) in Adx and AdxD animals by cimaterol. Calpastatin (the endogenous inhibitor of CDPs) activity was increased ($p < .05$) irrespective of glucocorticoid status. However, urinary NMH excretion was increased ($p < .05$) by Adx and was reduced ($p < .05$) by Dex treatment in control-fed animals, and cimaterol reduced ($p < .05$) urinary NMH excretion in Adx rats on day 7 of the study. Therefore, the changes in enzyme activities did not match the pattern and/or magnitude of urinary NMH excretion we detected suggesting other proteinases may be responsible for the elevated NMH excretion. Alternatively, the regulation of muscle protein degradation may be directed toward targeting or compartmentation of substrate rather than regulation of enzyme activities.

Interaction Between Glucocorticoid Status and β-adrenergic Agonists in Control of Muscle Growth and Protein Degradation

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INTRODUCTION

The synthesis and breakdown of proteins are functions common to most animal cells, and growth of muscle protein reflects a dynamic balance between protein synthesis and degradation. In the most general sense, growth occurs when protein synthesis exceeds protein breakdown. However, under some specific physiological and pathological circumstances muscle undergoes net protein breakdown due to a more rapid rate of degradation than of synthesis. Proteins in skeletal muscle, as in other cells, are continuously turning over, and the overall rate of protein breakdown in muscle, like the rate of protein synthesis, is precisely controlled. However, the mechanisms by which this is accomplished are not understood. It is believed that both protein synthesis and degradation play indispensible roles in determining steady state and fluctuations of protein concentrations in animal cells.

The regulation of both protein synthesis and proteolysis in muscle is important in overall energy homeostasis, in the control of muscle mass and body growth. Because skeletal muscle constitutes the major protein reservoir in the body and since hydrolysis of muscle proteins to generate amino acids is an important first step in gluconeogenesis, it is not surprising that overall rates of protein synthesis and degradation in muscle are

regulated by a number of hormones that are also critical in energy homeostasis (e.g. insulin and glucocorticoids).

Glucocorticoids are generally considered as catabolic hormones, but the mechanisms by which they mediate their catabolic effects in muscle are not completely known (Sharpe et al., 1986). In contrast, β-adrenergic agonists (e.g. cimaterol and clenbuterol) exert anabolic effects on muscle (Baker et al., 1984; Emery et al., 1984; Rothwell and Stock, 1988). β-adrenergic agonist could stimulate muscle growth either by enhancing protein synthesis (Emery et al., 1984) or by reducing muscle protein degradation (Forsberg and Merrill, 1986; Reeds et al., 1986). Given the catabolic nature of glucocorticoids, it is interesting that the glucocorticoids have a permissive effect on the growth-promoting actions of β-adrenergic agonists (Sharpe et al., 1986).

The objectives of this study, therefore, were to 1) re-evaluate the glucocorticoid-dependency for acute anabolic actions of dietary cimaterol on muscle growth, 2) examine effects of dietary cimaterol, different glucocorticoid status and their interactions on urinary N^{τ}-methylhistidine (NMH) excretion, an index of myofibrillar protein degradation,and 3) examine the relationship between the alterations of urinary NMH excretion with activities of cathepsin B, cathepsin D, cathepsin L and neutral proteinase and calcium-dependent proteinases (CDPs). Total protein synthesis, measured by incoporation of ^{14}-C tyrosine into isolated epitrochlearis muscle in vitro was also evaluated.

REVIEW OF LITERATURE

GENERAL FEATURES OF PROTEIN DEGRADATION

The quantitative balance between protein synthesis and protein degradation determines the net accumulation of protein in muscle. A fundamental concept that has been widely appreciated only within the past decade or so is the fact that all proteins in animal cells are continuously degraded and, for the most part, re-synthesized. The importance of the former process to a number of basic cellular functions has been thoroughly discussed in the past. On the other hand, degradation plays an indispensible role in enzyme regulation and cytoplasmic growth and remodeling, facilitates the removal of synthetic errors and other abnormalities of protein structure, and provides a source of free amino acids for essential metabolic reactions when the exogenous supply is limited (Mortimore, 1982).

About 5 to 10 percent of mature rat muscle protein is degraded per day (Allen, 1986). To maintain muscle mass, muscle would have to synthesize the equivalent amount of protein on a daily basis. When one considers the energetic costs of synthesizing one peptide bond (5 to 7 ATP) and the total number of peptide bonds that must be degraded and re-synthesized per day, it is easy to understand why protein turnover respresents a significant factor in the maintenance energy requirements of an animal (Allen, 1986). It is also easy to understand how the efficiency of growth or production could be enhanced if protein turnover could be altered in a favorable way. In growing animals, synthesis and

degradation rates are elevated with synthesis rate exceeding degradation rate; as maturity is reached both synthesis and degradation rates are decreased and ultimately reach a low and equal rate (Millward et al., 1976; McCarthy et al., 1983; Lewis et al., 1984).

Half-lives of protein molecules within a specific cell type differ widely from one protein to another. In liver, for example, they range from about 11 minutes in the case of ornithine decarboxlase to one or several days for the majority of cytosolic proteins (Mortimore, 1982). The variable half-lives of different proteins within one cell type indicate that protein degradation is not a random process. Rather it appears that proteins to be degraded are selected and that their subsequent rates of degradation vary from one to another.

The systems or organelles of a cell that are responsible for the degradation of cellular proteins must contain endoproteolytic and exoproteolytic activity. They may also contain other enzymatic activities to achieve the end point, namely complete hydrolysis. It is well established that two major pathways, lysosomal and extralysosomal proteolytic systems, exist in most cell types to account for the degradation of their intracellular proteins, the former involving proteolytic digestion within the lysosomes, and another involving digestion outside the lysosomes. However, the quantitative and functional significance of these proteolytic systems are not fully understood.

There is a rapid accumulation of literature on protein degradation especially regarding the characterization of cellular proteinases in various subcellular compartments and turnover of individual cellular proteins. Nevertheless, an inadequate mechanistic understanding of the biochemical details of protein synthesis and protein degradation --

especially degradation is blocking progress in research on the regulation of these processes. Nutritional and physiological experimentation has provided an important descriptive base, however, future progress is less likely to occur unless research is firmly grounded in the cellular and molecular biology of animal growth.

MECHANISMS OF INTRACELLULAR PROTEIN DEGRADATION

LYSOSOMAL PROTEOLYTIC PATHWAYS

Lysosomes are the most extensively-studied proteolytic system in mammalian cells. They contain a large number of acidic proteinases (cathepsins) as well as other acid hydrolases of which the cysteine proteinases (i.e. cathepsins B, H and L) are the most active (Bird et al., 1980; Goldspink and Lewis, 1985). Cathepsin D, an aspartyl proteinase also demonstrates moderate activity in some tissues including skeletal muscle (Matsumoto et al., 1983). Cathepsins are relatively small enzymes (20 to 30 KDa) and are present in virtually all mammalian cell types examined, with the exception of red blood cells (Beynon and Bond, 1986). Cathepsins are most active at the low pH of lysosomal environment and it is believed that their contribution to cellular proteolysis is associated only with lysosomes. However, pro-cathepsin B may have extralysosomal proteolytic functions associated with the processing of pro-hormones (e.g. pro-insulin) during migration of secretory granules (Docherty et al., 1982). Also, it is believed that cytosolic cystatins, the ubiquitous endogenous inhibitors of cysteine proteinases would prevent any significant extralysosomal cathepsin B-, H- or L-dependent proteolysis (Collela et al., 1986). The lysosomal apparatus is the major site for the breakdown of membrane proteins and glycoproteins, such as hormone receptors (Kettelhut et al., 1988). However, under poor nutritional conditions, many soluble proteins are also degraded in this organelle (Jefferson et al., 1974; Gronostajski et al., 1984; Furuno and Goldberg,

1986). For example, in muscle, liver, and other cells, the lack of insulin or of essential amino acids stimulates the uptake and hydrolysis of many cytosolic proteins within the enlarged lysosomes (autophagic vacuoles). It has been documented that in cultured fibroblasts (Gronostajski et al., 1984), perfused liver (Ward et al., 1979) and isolated rat muscle (Furuno and Goldberg, 1986), the lack of insulin or of serum leads to a two-fold increase in overall proteolysis which can be blocked by agents which block lysosomal acidification (e.g. chloroquine or methylamine) or by inhibitors of lysosomal thiol proteinases (e.g. leupeptin or E-64, both of which inhibit cathepsin B, H and L).

The cysteine proteinases belong to a family exhibiting sequence and structural homology. Other members of this family include Domain 2 of the CDPs (Suzuki, 1987) and the plant proteolytic enzyme, papain (Takio et al., 1983). Each of the cathepsins B, D, H and L is able to degrade myofibrillar proteins (Bird et al., 1980; Matsumoto et al., 1983; Goldspink and Lewis, 1985), especially denatured myosin; however, this does not prove that they indeed involve in the degradation of these proteins in vivo. Their activities have been assessed in a wide range of circumstances including aging (Goldspink and Lewis, 1985), muscular dystrophy (Kominami et al., 1984; Gopalan et al., 1987), chloroquine-induced myopathy (Sano et al., 1986), thyroid hormone-dependent muscle protein degradation (DeMartino and Goldberg, 1978), and glucocorticoid-dependent myopathy (Sohar et al., 1982), and in each case the activities of the cathepsins examined were altered appropriate to the acceleration in myofibrillar protein degradation either detected or expected.

CATHEPSIN B

Among the lysosomal proteolytic enzymes cathepsin B was the first identified and remains the most extensively studied. Cathepsin B exists as either a two-chain form (approximately 25 KDa and 4 KDa), a single-chain form (approximately 30 KDa) or both (Mason, 1986). In rabbit, ox and human, cathepsin B was purified as a mixture of two-chain and single-chain froms, whereas in sheep liver, cathepsin B existed only as a single-chain form (Mason, 1986). Cathepsin B was first sequenced in rat liver by Takio et al. in 1983. Recently, clones encoding a portion of the nucleotide sequence of rat cathepsin B were identified by San Segundo et al. (1985).

Cathepsin B is often assayed with benzoyl-Arg-2-naphthylamide as a substrate and more recently with benzylozxy carbonyl-Arg-Arg-methylcoumarylamide as substrate (Barrett and Keischke, 1981). Cathepsin B can act as an endopeptidase or as an exopeptidase (a polypeptidase) (Bond and Barrett, 1980); its action appears to depend on the substrate. For example, glucagon and native fructose 1,6-bisphosphate aldolase are good substrates for peptidyl-dipeptidase action (dipeptides are removed from the C-terminus of these substrates), where denatured hemoglobin, myofibrillar proteins and insulin B chain are degraded by the endoproteolytic action of cathepsin B (McKay et al., 1983). Cathepsin B also demonstrates limited proteolysis against native myosin and other myofibrillar proteins (Schwartz and Bird, 1977). All forms of cathepsin B, regardless of species or chain structure, have similar activities against the substrates Z-phe-Arg-NHMec and Z-Arg-Arg-NHMec and was inhibited by E-64 (Mason, 1986). Degradation

by cathepsin B was also inhibited by lack of reducing agent, iodoacetic acid, or leupeptin, but not pepstatin (Bird et al., 1980).

CATHEPSIN L

Cathepsin L, another lysosomal cysteine proteinase, purified from rabbit, rat, human, ox and sheep were all found to be two chain form with heavy chains of 22 KDa, 22 KDa, 25 KDa, 24 KDa and 24 KDa, respectively, and light chains of 8 KDa, 5 KDa, 5 KDa, 6 KDa and 6 KDa, respectively (Mason, 1986). The rabbit (Mason, 1986) and human (Mason et al., 1985) cathepsin L have their active site cysteine residing in the heavy chain. Cathepsin L is one of the most powerful lysosomal proteinases, based on its ability to hydrolyze myofibrillar protein (Bird et al., 1980) and azocasein (Mason et al., 1985). Purified cathepsin L from rat liver has a greater specific activity (10 times) against myosin than cathepsin B, and cathepsin L degrades myosin to peptides less than 5 KDa or to amino acids (Bird et al., 1980). It will also hydrolyze synthetic substrate such as carbobenzoxy-Phe-Arg-methylcoumarin (Kirschke and Barrett, 1985). All five species variants of cathepsin L mentioned earlier reacted in western blots with an antibody raised to the enzyme purified from human liver, showing that they share common antigenic sites (Mason, 1986). Complete amino acid sequences of cathepsin L have been documented by Ishidoh et al. (1987). They also cloned and sequenced a near full-length cDNA for rat cathepsin L.

CATHEPSIN D

Cathepsin D has several forms of similar molecular weight and different isoelectric points (Huang et al., 1980). The multiple forms of the enzyme appear to originate from a common high molecular weight precursor; limited proteolysis and other forms of post-translational processing result in the final forms identified (Hasilik and Neufeld, 1980; Beynon and Bond, 1986). The enzyme has limited activity against native proteins but considerable activity against denatured protein at pH 3.5 to 5.0. Like other proteinases of the aspartic family, cathepsin D is inhibited by pepstatin, diazoacetyl nor-leucine methyl ester, and epoxy nitrophenoxypropane (Beynon and Bond, 1986). It is similar to aspartic proteinases such as renin and pepsin in the amino acid sequence surrounding the Asp at the active site, Ile-Val-Asp-Thr-Gly-Thr-Ser. In addition, this family of proteinases preferentially attacks peptide bonds containing hydrophobic amino acids such as Phe-Phe, Phe-Tyr, Leu-Tyr (Beynon and Bond, 1986).

The amino acid sequence of porcine cathepsin D was reported by Shewale and Tang (1984). More recently human cathepsin D was cloned and sequenced by Faust et al.(1985).

Although the preceeding studies are supportive of a role for the lysosome in degradation of myofibrillar protein in vivo, other studies suggest otherwise. Myofibrils or myofilaments have not been observed in lysosomal structures in muscle, nor have lysosome-like organelles been observed in association with myofibrils (Allen, 1986). In addition, treatment of rats with lysosomal enzyme inhibitors failed to suppress myofibrillar

protein degradation, as indicated by urinary N^{τ}-methylhistidine (NMH) release, but reduced tyrosine release, an index of total muscle protein degradation (Lowell et al., 1986). Kadowaki et al. (1989) also reported that in diabetic and starved rats myofibrillar protein was preferenially degraded as indicated by urinary NMH, while in protein-deficient rats, non-myofibrillar protein degradation is selectively suppressed. These two studies provide strong evidence that the degradation of myofibrillar and non-myofibrillar proteins in skeletal muscle can be differentially regulated.

EXTRALYSOSOMAL PROTEOLYTIC PATHWAYS

It is now clear that significant proteolysis takes place in the cytosolic compartment of cells and it is important in the activation of post-translational processing and initiation of extensive degradation. Extralysosomal proteolytic pathways are thought to be more important to myofibrillar protein degradation than the lysosomal proteolytic pathway, among them, calcium-dependent proteinases (CDPs), neutral proteinase and ATP-dependent proteinase have received most attention.

CALCUIM-DEPENDENT PROTEINASES (CDPs)

Calcium-dependent proteinases (CDPs) have attracted many researchers' attention as possible initiators of the myofibrillar proteins degradation. In 1975 and 1976 Dayton et al. isolated a CDP from porcine skeletal muscle which was activated by millimolar concentration of calcium (mCDP). Because mCDP was the first proteinase found that was endogenous to striated muscle cells and had ability to degrade intact myofibrils at physiological ionic strength and pH values, it was suggested (Dayton et al., 1975; 1976) that this enzyme initiated myofibrillar proteins degradation by dissassembling these proteins from their highly organized structure in the myofibril. Substantial evidence has accumulated to support this suggestion, but the exact physiological role of mCDP remains unknown.

In 1981 Dayton et al. and Szpacenko et al. reported the presence of another CDP

which was activated by micromolar concentrations of calcium: μCDP. Both CDP isozymes have been isolated from various tissues and cells of mammals. The concentration of Ca^{2+} for 50 % activity is 2 to 75 μM for μCDP and 0.2 - 0.8 mM for mCDP (Suzuki et al., 1984). Their properties, other than calcium sensitivity, are quite similar. Calcium-dependent proteinases are fully active in the neutral pH region only in the presence of reducing reagents such as mercaptoethanol (Suzuki et al., 1987). The activity of calcium-dependent proteinase is inhibited by thiol group-modifying reagents and Ca^{2+}-chelating reagents (e.g. EGTA). Leupeptin, antipain, and E-64 (an epoxysuccinyl derivative) are potent inhibitors (Parkes et al., 1985). Limited proteolysis is a typical feature of CDPs (Suzuki, 1987). It generally hydrolyzes protein substrates only to large fragments, not to small peptides or amino acids. It has no strict requirement for the sequence of the cleavage sites, although a relative preference for large hydrophobic residues such as leucine and valine in the P2 site is suggested (Sasaki et al., 1984).

In recent years dramatic progress has been made in understanding the biology of μCDP and mCDP. Both enzymes consist of two subunits; their catalytic 80 KDa subunits share 50 % sequence homology and a 30 KDa regulatory subunit which is identical in both enzymes (Emori et al., 1986 a,b; Aoki et al., 1986; Suzuki, 1987). The 80 KDa subunit is composed of four distinct domains (I,II,III and IV) of 80, 240, 240 and 144 amino acid residues, respectively (Minami et al., 1987; Suzuki, 1987). The domain II is responsible for proteinase activity and has amino acid sequence homology with cysteine proteases (i.e. cathepsin B, H and L and papain; Suzuki, 1987). The C-terminal domain

IV has sequence homology with the four calcium-binding EF-hand structures of calmodulin (Minami et al., 1987; Suzuki, 1987). No clear sequence homology with known protein sequences have been found in domains I or III, and the functions of these domains are not yet clear (Suzuki, 1987). Since the smaller subunit (30 KDa subunit) is identical and functionally interchangeable for μCDP and mCDP (Suzuki, 1987), indicating that the difference in Ca^{2+}-sensitivity can be ascribed to structural differences in the 80 KDa subunits (Suzuki, 1987).

The 30 KDa subunit of μCDP and mCDP in rabbits consists of 266 amino acid residues with 2 domains. The N-terminal domain (64 residues) has 50 % glycine residues with the remaining residues being mostly hydrophobic (Emori et al., 1986 b). This portion of protein may interact with membranes or perhaps other substrates (Emori et al., 1986 b); hence, it may be involved in targeting of actions of the CDPs. The C-terminal domain (168 residues) is 50 % homologous to the C-terminal domain IV of the 80 KDa subunit with four calcium-binding EF-hand structures (Suzuki, 1987).

For many years the biological signficance of mCDP was questioned since intracellular concentration of calcium never reached the concentration required for its activation. Recently, however, both forms of the enzyme were found to undergo calcium-dependent autolysis (Suzuki et al., 1984) which removed about 20 and 90 residues from the large and small subunits, respectively (Suzuki et al, 1987). This modification increases sensitivity of both enzymes to calcium. Eventually, autolysis inactivates mCDP and may limit the extent of its activity (Mellgren, 1987). However, the calcium requirement of mCDP may still exceed a physiologically attainable concentration

(Mellgren, 1987). Hence, the role of mCDP could be in the degradation of membrane proteins where fluxes of calcium ions at localized sites of the cell membrane may be sufficient to activate the enzyme (Mellgren, 1987). This would imply the participation of the μCDP and mCDP in different processes during calcium-dependent proteolysis and possibly that μCDP may have greater significance to the degradation of myofibrillar proteins than mCDP.

Like other cellular proteinase, an endogenous proteinase inhibitor, calpastatin (CDPI) that specifically inhibits CDPs co-exists with CDPs in the cytosol (Parkes, 1986). Calpastatin is CDP-specific and is present in tissues in three to thirty-fold excess of that required for complete inhibition of tissue CDP activity (Gopalakrishna and Barsky, 1986). Despite the excess of calpastatin, it was located in the cytosol and that the binding of CDPs to their substrates reduced CDPs susceptibility to inhibition. Therefore, the presence of calpastatin in the cytosol may prevent indiscriminate degradation of cytosolic proteins (Gopalakrishna and Barsky, 1986; Suzuki et al, 1987).

ATP-DEPENDENT PATHWAY

In most cells, an energy-dependent proteolytic process can be demonstrated as ATP depletion leads to a dramatic fall in protein breakdown (Goldberg and St.John, 1976). Most of our knowledge about this proteolytic process comes from studies of reticulocyte extracts, but its general features appear similar in all eukaryotic cells (Kettelhut et al., 1988). Measurement of such an ATP-dependent pathway in incubated skeletal muscles has only recently been possible, and has required some new techniques (Han et al., 1988). Skeletal muscle, like other tissues, contained the soluble ATP-dependent ubiquitin-dependent proteolytic pathway (Fagan et al., 1987). In this process, the protein substrate is initially conjugated to a heat-stable 9 KDa polypeptide, ubiqutin (Ciechanover et al., 1984). This covalent ligation process attaches the carboxyl terminal of ubiquitin to epsilon-amino groups on lysine residues in the protein and involves three or four distinct enzymatic steps, and requires ATP (Ciechanover et al., 1984). Ubiquitin-conjugation tags the protein conjugates for subsequent hydrolysis by a very large enzyme complex called the Ubiquitin-Conjugate-Degrading-Enzyme, or UCDEN (Ciechanover et al, 1984). This multi-component cytosolic pathway is known to catalyze the selective degradation of proteins with highly abnormal structures, many short-lived regulatory proteins, and the bulk of cell proteins during optimal growth (Lowell et al., 1986). We have very little information about the contribution of this system relative to the lysosomal system or other systems in the degradation of the myofibrillar proteins in most cells, and its regulation at

pre- and post-translational levels has not been evaluated.

NEUTRAL PROTEINASE

This proteinase was first isolated from rat intestinal smooth muscle and was shown to be a trypsin-like serine proteinase which was active at neutral pH (Beynon and Kay, 1978). The significance of the neutral proteinase to myofibrillar protein degradation has received little attention in recent years. Neutral proteinase is able to degrade various myofibrillar proteins at neutral pH (Kar and Pearson, 1980; Kay et al., 1982). A study by Kar and Pearson (1980) suggested that neutral proteinase may be important in conditions when muscle protein degradation is accelerated. These authors found that the activity of neutral proteinase in skeletal muscle was accelerated greatly in Duchenne dystrophy, polymyositis and in denervating diseases.

The cooperation of CDPs and neutral proteinase is thought to be important to myofibrillar protein degradation (Kay et al., 1982; Pontremoli and Melloni, 1986). These authors found both CDPs and neutral proteinase are capable of initiating myofibrillar protein degradation by disassembly of Z-line structure. Although a specific inhibitor of neutral proteinase has been isolated from smooth muscle and characterized (Carney et al., 1980), the existence of this inhibitor in skeletal muscle has not been estabilished.

ENDOCRINE REGULATION OF PROTEIN DEGRADATION IN SKELETAL MUSCLE

Many metabolic hormones influence muscle protein turnover. To whole-animal endocrinologists, the primary anabolic hormones are pituitary growth hormone and insulin, while the thyroid hormones and the glucocorticoids have significant catabolic actions (Florini, 1987). In cultured cells a striking stimulation of muscle cell proliferation has been obtained with the somatomedins, or insulin-like growth factors, but growth hormone is generally inactive (Florini, 1987). Although glucocorticoid is well-estabished as a catabolic hormone in intact animals, a glucocorticoid is a component of nearly all serum-free media for the growth of muscle cells in culture.

In recent years, β-adrenergic agonists have been shown to exert an anabolic effect on skeletal muscle growth (Baker et al., 1984; Emery et al., 1984; Rothwell and Stock, 1988). Since this thesis will focus on the effects of glucocorticoid status and β-adrenergic agonists on muscle growth, a review of their actions is provided below.

EFFECTS OF β-ADRENERGIC AGONISTS

β-adrenergic agonists have received a great deal of attention in recent years. One of these -- clenbuterol -- was originally designed as a respiratory drug but was subsequently shown to have a stimulatory effect on rat growth. Since then, it has been used to stimulate growth and feed efficiency in poultry, sheep, and cattle (Dalrymple et al., 1984; Baker et al., 1984; Ricks et al., 1984, respectively). Most of the reseachers in this area are currently trying to elucidate the mechanisms by which β-agonists mediate growth. Several studies have demonstrated that β-adrenergic agonists, either directly or indirectly, reduce skeletal muscle protein degradation. Li and Jefferson (1977) and Forsberg and Merrill (1986) have reported that isoproterenol and cimaterol reduced tyrosine release from isolated rat skeletal muscle and monolayer cultures of rat myotubes, respectively. Reeds et al. (1986) and Bohorov et al. (1987) reported that feeding clenbuterol stimulated muscle growth in rats and sheep, respectively, and determined that the hypertrophy resulted from a reduction in fractional rates of protein degradation rather than from an increase in protein synthesis. Zeman et al. (1987) however, observed that muscle protein synthesis is increased with no effect on fat by treatment of rats with clenbuterol.

Recently, some investigators have attempted to understand the mechanisms by which the β-adrenergic agonists stimulate muscle protein gain. Wilson et al. (1988) reported that dietary cimaterol increased acetate oxidation in isolated muscles and stimulated transport of amino acids via a sodium gradient-dependent process, and Helferich et al. (1988)

reported that ractopamine increased muscle α-actin biosynthesis in swine. It is known that both denervation and hind-limb suspension decrease content of rRNA, α-actin mRNA, and cytochrome C mRNA in adult rat skeletal muscle (Babij and Booth, 1988). However, the provision of clenbuterol to adult female rats during a 7-day period of denervation of the soleus and gastrocnemius muscle prevented entirely the loss of rRNA, α-actin mRNA, and cytochrome C mRNA that normally occurs in denervated muscle (Babij and Booth, 1988). These authors suggested that clenbuterol can maintain the expression of certain RNAs in atrophying adult rat skeletal muscle.

Forsberg et al. (1989) reported that the enhancement of rabbit muscle weight by dietary cimaterol was associated with an increase in total DNA and with a reduction in myofibrillar protein degradation as indicated by urinary NMH excretion. They also found that cimaterol did not affect activities of cathepsin B, cathepsin D or neutral proteinase but reduced activities of the mCDP and μCDP by 58 % and 57 %, respectively. These data implied that cimaterol-dependent myofibrillar protein accretion was mediated, at least in part, by a reduction in myofibrillar protein degradation and the reduction of CDPs activities is likely associated with the reduction of myofibrillar protein degradation. To date, the cellular and molecular mechanisms underlying the growth promotion of β-adrenergic agonists are not clear.

EFFECTS OF GLUCOCORTICOIDS

Glucocorticoids are generally regarded as protein catabolic hormones. In the intact animal, elevated levels of glucocorticoids lead to a net loss of muscle proteins and conversion of amino acids into carbohydrates. Indeed, this is a major endocrine response to starvation, allowing the body to maintain essential glucose levels in the brain while metabolizing available fat and protein stored in other tissues such as muscle. Overall the glucocorticoids cause a sparing of glucose and a tendency towards hyperglycaemia. There is a reduction in glucose uptake in muscle, skin, adipose and lymphoid tissues and increased protein catabolism in these tissues resulting in a release of amino acids (Sharpe et al., 1986). In addition, glucocorticoids stimulate lipolysis in adipose tissue leading to an increase in circulating fatty acid and glycerol concentrations (Sharpe et al., 1986).

It is well-established that large doses of glucocorticoids reduce growth rate and cause muscle atrophy (Sharpe et al., 1986), but the physiological role of normal concentrations of glucocorticoids is not clear. Young (1980) observed that growth in adrenalectomized rats was not affected by a physiological replacement dose of glucocorticoid. Furthermore, only when the plasma concentration of corticosterone was elevated to levels associated with "stress" responses was growth rate reduced. This reduction in growth rate was associated with increased myofibrillar protein breakdown as determined by the estimate of urinary NMH excretion. In contrast, a recent study by Sillence et al. (1985) with the compound trilostane, an inhibitor of adrenal corticosterone production in young female rats, has shown that reduction in plasma corticosterone concentrations was associated with

an increase in growth rate and feed conversion efficiency. These results imply that at normal physiological concentrations the glucocorticoids are exerting an inhibiting effect on growth. Goldberg et al. (1980) also reported that physiological levels of glucocorticoids may inhibit protein and DNA synthesis, since these processes increased in rat muscle after adrenalectomy.

Barnett and Star (1981) carried out a growth trial on sheep and found a significant negative correlation between growth rate and free cortisol concentration while no significant correlation was detected when total cortisol concentration was assessed instead of free cortisol. Many studies have confirmed that administration of a phamacologic dose of glucocorticoids reduce muscle protein synthesis both in vivo (Rannels and Jefferson, 1980; Odedra et al. 1983) and in vitro (McGrath and Goldspink,1982); Odedra et al.(1983) also suggested that myofibrillar proteins are preferentially inhibited during glucocorticoid administration. Furthermore, protein synthesis is reduced due to a reduction in DNA synthesis (Sharpe et al 1986), reduced RNA content of the muscle (Rannels and Jefferson, 1980) and also due to reduced efficiency of protein synthesis (i.e. protein synthesis per unit RNA is reduced). As pointed out by Rannels and Jefferson (1980), glucocorticoids were interfering in the initiation step of protein synthesis.

The effects of glucocorticoids on protein degradation, however, have been controversial. It appears that the response in muscle protein degradation to glucocorticoids is dependent upon mode of administration (e.g. subcutaneous injection are more effective than intraperitoneal injection, Santidrian et al., 1981) and duration of glucocorticoid administration. Odedra et al. (1983) reported that pharmacologic doses of

glucocorticoids stimulated rat myofibrillar protein degradation for 2 - 4 days after which basal levels of protein degradation were restored. Santidrian et al. (1981) also observed a similar time course of effects when degradation rate was estimated by NMH excretion. To futher emphasize the complex nature of the relationship between glucocorticoids and growth, Sharpe et al. (1986) reported that glucocorticoids are necessary for the growth-promoting and repartitioning actions of β-adrenergic agonists. In their study clenbuterol stimulated weight gain, increased the proportion of muscle (estimated as g gastrocnemius muscle.100 g^{-1} body weight) and reduced the proportion of fat (estimated as g epididymal fat pad.100 g^{-1} body weight) in the control animal. But clenbuterol appeared to have no effect on any of the three growth parameters measured in adrenalectomized rats. Giving dexamethasone therapy to restore the physiological levels of glucocorticoids restored the growth-promoting action of clenbuterol. Since treatment with clenbuterol in control animals caused no change in total free or bound corticosterone concentrations, it was suggested that clenbuterol is not acting through the glucocorticoids but rather that glucocorticoids exert a permissive effect on the growth-promoting actions of the β-adrenergic agonists.

MATERIALS AND METHODS

This experiment was conducted in two blocks with twenty four animals in each block. Forty eight male Sprague-Dawley rats weighing 250 g were received either adrenalectomizied or sham-operated from Charles River Breeding Laboratories, Inc. (Wilmington, MA). On the day of arrival, animals were placed in individual stainless steel cages and housed in a temperature-controlled room with a 12-hr light - 12-hr dark cycle. Powdered control diet (Table 1) and water (containing .85 % NaCl; w/v) were provided ad libitium for two days before they were assigned to one of the following six experimental treatments :

1) Sham-adrenalectomized

2) Sham-adrenalectomized + dietary cimaterol (25 mg/kg)

3) adrenalectomized

4) adrenalectomized + dietary cimaterol (25 mg/kg)

5) adrenalectomized + dexamethasone (1.2 μg/100g BW/day)

6) adrenalectomized + dexamethasone (1.2 μg/100g BW/day)
+ dietary cimaterol (25 mg/kg)

Two days after arrival, four rats were randomly assigned by weight to each experimantal treatment such that average initial weights between treatments were as close as possible. Animals were placed in individual cages with the control diet or cimaterol

containing diet and water (containing .85 % NaCl; w/v) available ad libitum. Dexamethasone (Dex; 1.2 μg/100g BW/day) was dissolved in corn oil and was administered subcutaneously twice daily, once in the morning once in the evening with 12 hours apart. Animals not receiving Dex were given twice daily injections of corn oil.

Animals were weighed daily following injections. Daily feed intake and daily water intake were also monitored. Total 24 hour urinary output from each rat on days 1, 2, 4 and 7 was collected in the presence of 1 ml of 4 N HCl to prevent bacteria degradation of urine. Total volume of daily urinary excretion was determined and an aliquot was saved for N^{τ}-methylhistidine (NMH) determination.

Table 1. AIN-76 purified Diet

Ingredient	%
Casein	20.0
DL-Methionine	.3
Cornstarch	15.0
Sucrose	47.4
Fiber	2.4
Corn oil	5.0
AIN Mineral mix	3.5
AIN Vitamin mix	1.0
Choline bitartrate	.2
Corn cob*	5.2

* Corn cob premixed with cimaterol (480 ppm) was used for cimaterol containing diet.

On the eighth day of the study, animals were anesthetized with halothane and euthanized by exsanguination. Immediately following death two epitrochlearis muscles were removed from each carcass with tendons attached for assaying protein synthesis as

described below. Tissues including left hind-limb muscles , liver , heart and kidney were removed and weighed and frozen at -80°C. Total DNA and RNA concentration as well as activities of cathepsin B, D, L and neutral proteinase and calcium-dependent proteinases were assessed in left lower hind-limb muscles and livers.

Assessment of protein synthesis. Epitrochlearis muscle samples were attached by their tendons to a plastic ring fitted with two stainless steel clips, which maintained the muscle samples at their resting lengths, and were incubated in 3.5-cm Corning plastic tissue culture dishes containing 4 ml of Krebs-Henseleit bicarbonate medium for two hours. Incubation was conducted on a rocking platform (30 oscillations/min) within a humidified water-jacketed CO_2 incubator (5 % CO_2: 95 % air) at 37°C. After which samples were removed from their frames, rinsed with ice-cold phosphate-buffered saline and homogenized in distilled water. Tissue protein was precipitated by the addition of 3 % trichloroacetic acid (TCA; final concentration, w/v) and recovered by centrifugation. The protein-containing pellet was washed twice with 3 % TCA and solubilized with 1 ml of Protosol (Du Pont). Tyrosine conversion to protein was determined by the ^{14}C-activity associated with the washed protein pellet using liquid scintillation counter (Packard Model 2425). Ability of epitrochlearis muscle to convert tyrosine to protein was used as an index of protein synthesis.

Assessment of urinary NMH. A 2.5 mM NMH standard was prepared and processed identically to urine samples. Urine or NMH standards were combined with 2 ml 4 N HCl and heated at 100°C to deacetylate NMH. Samples (.5 ml) of deacetylated NMH were lyophilized in disposable 10x75 mm glass tubes in a Virtus Uni-Top 600 SL

freeze dryer for 12 hours then resuspended in .5 ml of a 7:1:1:1 mixture of ethanol:phenylisothiocyanate:triethylamine:distilled deionized water, respectively, and allowed to stand at room temperature for 20 minutes. Samples were again lyophilized then resuspended in .5 ml of a 5 mM phosphate buffer (pH 7.4) containing 5 % (v/v) acetonitrile. The sample was taken up in a 1 ml disposable plastic syringe and filtered through a Gelman Science Acro LC3A .45 micron filter.

The sample was placed in a limited volume insert for injection of 50-100 μl into HPLC system. The HPLC system consisted of two Model 510 solvent delivery pumps, a model 680 gradient controller, a WISP 710 B auto-injector, a model 441 UV-detector fitted with a 254 nm filter, a model 730 integrating recorder, a 15 cm Pico-Tag amino acid analysis column, and a model TCM column heater, all manufactured by Waters Associates. Solvents used were Eluent 1 and Eluent 2 from Waters Associates with Pump A and Pump B delivering Eluents 1 and 2, respectively. Solvent delivery conditions and gradients used for separation of NMH from other urinary PITC-reactive components are shown in Table 2. Column temperature was maintained at 44°C.

Assessment of DNA concentration. DNA content of muscle and liver were determined according to the method of Labarca and Paigen (1980) using Hoechst compound 33258 (Polysciences) as a fluorescent marker of DNA and calf thymus DNA as a standard. Tissue (.5-.8 g) was homogenized in 6 volumes of distilled deionized H_2O in 25x150 mm glass tubes on ice, using a Polytron (Kinematica) large head at 70 % power for 15 sec and 25 sec for liver and muscle, respectively. Homogenate (.1 ml) was placed in 15x85 mm tubes containing 2.0 ml phosphate buffered saline (.05 M $NaPO_4$,

2.0 M NaCl, pH 7.4) and 2.2 ml Hoechst dye reagent so that final concentration of Hoechst dye was 1 μg/ml. The mixture was vortexed and was read on a fluororesence spectrophotometer (Perkin-Elmer 650-10S) at excitation 360 nm ,emission 418 nm.

Table 2. Conditions used for isolation and quantitation of NMH by high performance liquid chromatography

Time(min) Initial	Flow (ml/min)	Percent Eluent 1	Percent Eluent 2	Gradient Cruve *
Initial	1.0	100	0	-
5.0	1.0	97	3	11
15.0	1.0	94	6	5
18.0	1.0	91	9	8
18.5	1.0	0	100	10
28.5	1.0	0	100	6
29.0	1.0	100	0	6

* Gradients refer to program gradient profiles available on Water's Model 680 Automated Gradient Controller.

Assessment of total RNA. Total RNA was determined according to the method of Monro and Fleck (1969). Tissue (.5 g) was homogenized in 6 volumes of distilled and deionized H_2O. A portion of this homogenate (1.5 ml) was placed in a 15-ml centrifuge tube with 1.5 ml ice-cold 20 % TCA (w/v). The mixture was swirled immediately and was stood on ice for 1 hour and was centrifuged at 5000 g at 5°C for 10 minutes. The pellet was resuspended in 2 ml ice-cold 10 % TCA (w/v) and was centrifuged as previouly described. This washing procedure was repeated once. Supernatant was discarded. Two ml of 5 % TCA (w/v) were added and the pellet was resuspended with a glass rod. This suspension was heated at 90°C for 15 minutes and then was centrifuged

at 2000 g for 5 minutes. A portion of final supernatant (.1 ml) was mixed with .4 ml of 5 % TCA and 3 ml of Orcinal reagent (Sigma O-1875) and was heated at 95°C for 30 minutes. The mixture was cooled to room temperature, and its absorbsance versus 0 μg RNA blank was determined by using a spectrophotometer (Shimadzu UV-160). Standard curves of RNA and DNA were established by using Escherichia coli RNA (Sigma R-1753) and calf thymus DNA (Sigma D-1501), respectively. The absorbance of each sample was substracted by absorbance caused by DNA according to DNA content of each sample.

Cathepsin B. Cathepsin B activity was measured by the method of Barrett and Kirschke (1981) which measures the release of β-naphthylamine fluorometrically from CBZ-alanyl-arginyl-arginyl-methoxy-β-naphthylamine (Enzyme systems products Ltd., Livermore, Ca). Samples of muscle or liver were homogenized in 6 volume of ice-cold buffer (pH 6.0) containing 155 mM $KH_2PO_4 3H_2O$, 4.5 mM citric acid and 4 mM ethylenediaminetetraacetic acid (EDTA) by using polytron for 30 seconds on setting 7.0. The sample was kept on ice at all times to minimize degradation of cathepsin B by other proteolytic enzymes. Tissue homogenate (40 μl) was mixed with 170 μl distilled H_2O, 500 μl of buffer prepared as previously described and 250 μl of 2 mM dithiothreitol, then equilibrated for 10 minutes at 37°C in a water bath. Forty μl of 5 mM CBZ-alanyl-arginyl-arginyl-methoxy-β-naphthylamine in Dimethyl sulfoxide was added. Muscle samples were incubated for 100 minutes and liver for 1 hour, in glass tubes at 37°C in a water bath. Reactions were stopped by adding 2 ml 1 N HCl. Samples were kept on ice for one hour and centrifuged at 1000 g for 12 min. and fluorescence of the released β-

naphthylamine (excitation 292 nm, emission 410 nm) within supernatant determined using a fluorescence spectrophotometer (Perkin-Elmer 650-10S).

Neutral proteinase assay. Muscle or liver (1 g) was homogenized in 6 volumes of distilled and deionized H_2O followed the procedure as described in cathepsin B assay. A portion of tissue homogenate (300 μl) was placed in 15x85 mm culture glass tube. Tissue homogenate was mixed with 2.55 ml 50 mM Tris buffer (pH 7.5) and vortexed to resuspend homogenate. Substrate solution (150 μl) containing 10 mM of CBZ-glycyl-glycyl-argiyl-β-methoxynaphthylamine in dimethyl formamide was added. Each sample had one control and three triplicates, was incubated in 37°C water bath for 2 hrs whereas control was iced immediately to stop the reaction. After 2 hrs incubation, the sample was iced for 2 hours to precipitate protein and centrifuged at 3000 g for 12 minutes. The fluoresence of released β-naphthylamine (excitation 292 nm, emission 410 nm) was determined with a fluorescence spectrophotometer (Perkin-Elmer 650-10S).

Cathepsin D assay. Cathepsin D activity was determined according to the method of Takahashi and Tang (1981) using bovine hemoglobin as a substrate. Samples were homogenized in 6 volumes of ice-cold distilled and deionized H_2O follow the procedure described previously. Sample homogenate (.1 ml) was mixed with 1.9 ml of 0.25 M sodium formate buffer (pH 3.2) and 0.5 ml hemoglobin substrate (5 %; w/v). Reaction mixtures were incubated at 37°C for 20 minutes after which 2 ml of 10 % TCA was added to stop reaction. Supernatant was filered through No.50 Whatman paper and absorbance at 280 nm was measured by spectrophotometer (Shimadzu UV-160). Incubation was conducted in triplicate with one control for each sample.

Cathepsin L assay. Cathepsin L activity was determined according to the method of Barrett and Kirschke (1981) using azocasein (Sigma) as a substrate. Samples were homogenized in 6 volumes of distilled and deionized H_2O. Tissue homogenate (0.25 ml) was mixed with 0.25 ml buffer A (0.1 M sodium acetate - acetic buffer pH 5.0, 1 mM EDTA, 40 mM cysteine (Sigma), 0.1 % pepstatin; w/v). After 5 minutes on the bench for activation, 0.5 ml of azocasein-urea substrate solution (2 % azocasein ; w/v and 6 M urea dissolved in buffer A without cysteine) was mixed in, and the reaction was allowed to proceed for 30 minutes at 40°C. At end of incubation, 5 ml of 3 % TCA (w/v) was introduced to stop the reaction, and the reaction mixture was filtered through 7-cm Whatman No.1 paper. The absorbance of the filtrate was determined at 366 nm by spectrophotometer (Shimadzu UV-160). Blanks are prepared in which TCA was added immediately after the tissue homogenate.

CDPs and calpastatin assay. Activities of CDPs and calpastatin were measured by the method of Gopalakrishna and Barsky (1986). Muscle samples weighing 1.5 g were homogenized in 5 to 10 volume of 50 mM Tris-HCl (pH 7.5) containing 1 mM EDTA, 10 mM β-mercaptoethanol, and 150 nM pepstatin A (Sigma), and were centrifuged at 6,000 g for 30 minutes. A portion of supernate (0.5 ml) was saved for calpastatin assay. To the rest of supernate, 10 μl of 1 mM leupeptin (Sigma), 1 ml of phenyl-Sepharose beads (Sigma) which was previously equilibrated with buffer A (20 mM Tris-HCl, pH 7.5, 0.1 mM $CaCl_2$, 10 mM β-mercaptoethanol, 20 μM leupeptin) containing added 0.25 M NaCl, and 0.3 ml of 5 M NaCl were added. After shaking the mixture for 5 minutes, 0.2 ml of 0.1 M $CaCl_2$ was added and the shaking was continued for another 10 minutes.

Then this gel suspension was poured into a column (1 x 8 cm). The packed column was then washed successively with 2 ml of buffer A with added 0.25 M NaCl, buffer A without NaCl, and buffer A without leupeptin. Then the Ca^{2+}-dependently bound mCDP and μCDP were eluted successively with 4 ml of buffer B (20 mM Tris-HCl, pH 7.5, 1 mM EGTA, 10 mM β-mercaptoethanol) with added 0.1 M NaCl and 4 ml of buffer B without NaCl.

Proteinase activity was determined using alkali-denatured casein as substrate. The reaction mixture containing 4 mg of Hammerstan casein (EM Sci.) in 20 mM Tris-HCl buffer, pH 7.5, 2 mM dithiothreitol, and 0.05 mM $CaCl_2$ (for μCDP) or 1 mM $CaCl_2$ (for mCDP), along with 0.6 ml of CDP extracts, in a total volume of 0.8 ml, was incubated at 25°C for 30 minutes. The reaction was terminated by adding 0.2 ml of 36 % TCA. The TCA-soluble digestion products were measured using Bio-Rad protein assay dye reagent was added to 0.8 ml of the TCA-soluble fraction, and the absorbance was read at 595 mm after 10 minutes using Shimadzu UV-160 spectrophotometer. One unit of CDP activity was defined as the amount of enzyme which caused an increase of 1/10 absorbance unit at 595 nm after 30 minutes incubation at 25°C.

Calpastatin assay. Calpastatin activity was assayed on the basis of its inhibition of mCDP activity. The supernate saved from CDP assay was boiled for 10 minutes, and were centrifuged at 15,000 g for 10 minutes. This heated supernate (80 μl) was mixed with 400 μl CDP extracts and 20 μl of 0.1 M $CaCl_2$. After 5 minutes incubation at room temperature, 0.3 ml of casein substrate solution was added and the incubation was continued for another 30 minutes at 25°C. The reaction was terminated by adding 0.2 ml

of 36 % TCA. Blanks were prepared as described in CDP assay. One unit of calpastatin was defined as the amount of enzyme which caused a disappearance of one unit mCDP.

Statistical analysis. This experiment was a randomized block design. There were two blocks in this experiment with six treatments in each block. Data were analyzed by using analysis of variance. Differences among individual means were tested for significance by using SNK multiple range test. A significance level of 5 percent was used for all comparisons (Petersen, 1985).

RESULTS

Adrenalectomized-rats had a lower starting weight than rats that had received sham-operations. This was due to the requirement for observation and shipping of animals post-surgery (Table 3 and Figure 1).

Effect of glucocorticoid status and cimaterol on rat growth, feed and water intake,feed efficiency and urine execretion are shown in Table 3. Dietary cimaterol increased ($p > .05$) weight gain in sham-adrenalectomized rats by 10.6 %. This was accompanied by a 7.4 % decrease in feed intake. Adrenalectomy (Adx) reduced ($p < .05$) feed intake and weight gain of control-fed animals by 11.5 % and 20.7 %, respectively. Cimaterol only slightly increased the weight gain of adrenalectomized rats (5.6 %; $p > .05$) but decreased their feed intake ($p < .05$). Weight gain in adrenalectomized rats receiving dexamethasone treatment (AdxD) was further decreased ($p < .05$) by 19.6 %, compared to their adrenalectomized counterparts. Their feed intake, however, increased ($p < .05$) 15.3 %. When AdxD rats were supplemented with cimaterol, their feed intake decreased ($p < .05$) 14.9 % but the weight gain increased 46.8 % ($p < .05$), compared to control-fed rats (Figures 2 and 3).

Feed efficiency was not affected by ADX but was reduced ($p < .05$) in AdxD rats when compared to sham-controls. Cimaterol did not affect the feed efficiency of sham-controls; however, it improved ($p < .05$) feed efficiency of Adx and Adxd animals (Figure 4).

Table 3. Effect of glucocorticoid status and cimaterol on average daily gain, water intake and urine excretion in rats[1].

	Experimental Treatment					
	Sham	Sham+cim	Adx	Adx+cim	AdxD	AdxD+cim
Initial weight (g)	294.8±12.2^{a}	296.9±8.7^{a}	265.8±21.9^{b}	268.5±10.2^{b}	268.4±10.6^{b}	264.1±12.4^{b}
Final weight (g)	352.8±20.6^{a}	362.5±14.2^{a}	312.9±23.7^{b}	315.0±28.1^{b}	307.4±11.2^{b}	318.6±14.8^{b}
Average daily intake (g/day)	24.4±2.0^{a}	22.6±1.0^{ab}	21.6±1.2^{b}	17.6±3.3^{c}	24.9±1.9^{a}	21.2±1.3^{b}
Average daily gain (g/day)	8.5±.5^{ab}	9.4±1.3^{b}	6.7±.9^{c}	7.1±1.8^{ac}	5.4±1.1^{d}	7.9±1.2^{ac}
Feed/gain ratio	2.9±.1^{ab}	2.4±.3^{a}	3.3±.3^{b}	2.5±.2^{a}	4.8±1.0^{c}	2.7±.4^{ab}
Water intake (ml/day)	33.7±7.5^{a}	31.8±6.5^{a}	67.9±13.9^{b}	48.9±11.6^{c}	73.6±11.0^{b}	63.3±9.4^{b}
Urine excretion (ml/day)	15.6±5.9^{a}	10.5±4.5^{a}	43.6±11.0^{bc}	25.0±9.1^{d}	46.7±10.2^{b}	36.8±7.2^{c}
Water balance (ml/day)	18.1±2.9^{a}	21.3±3.1^{ab}	24.3±4.3^{bc}	23.9±3.7^{bc}	27.0±4.2^{c}	26.5±3.2^{c}

[1] Values are means±SE. Values in the same row with differing superscripts differ significantly.

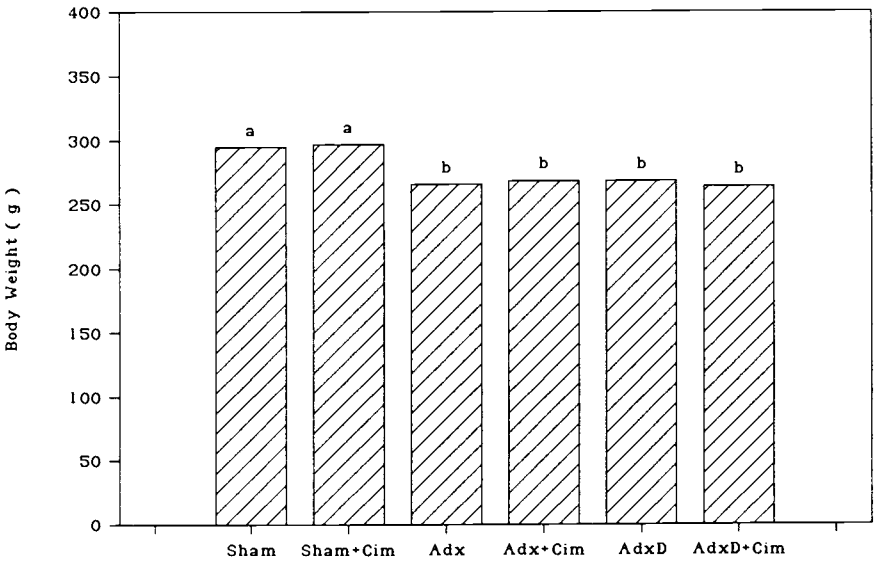


Figure 1 Initial body weight.

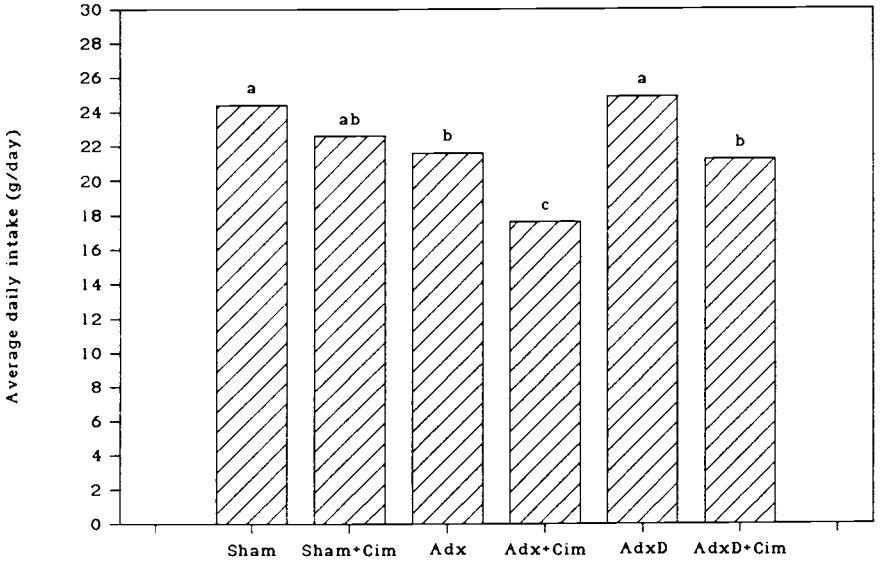


Figure 2 Effect of glucocorticoid status and cimaterol on average daily feed intake.

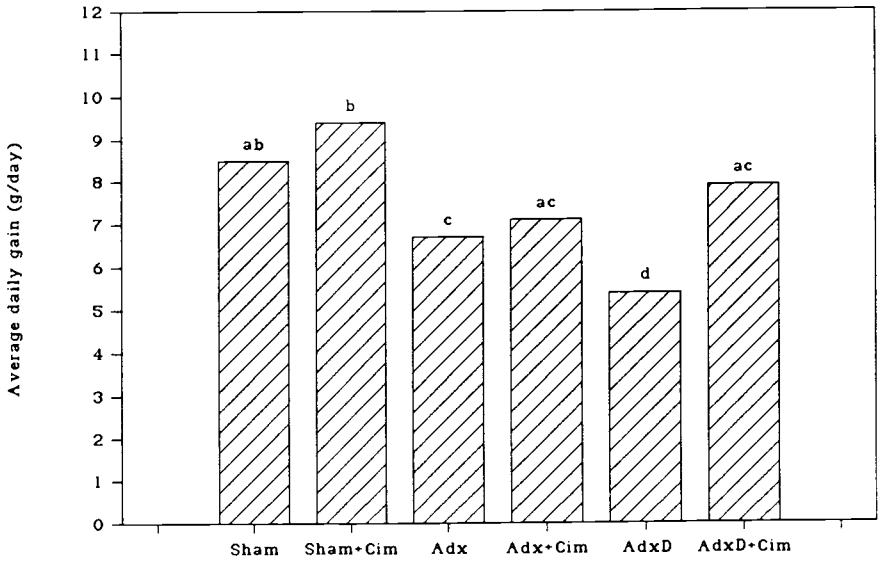


Figure 3 Effect of glucocorticoid status and cimaterol on average daily gain.

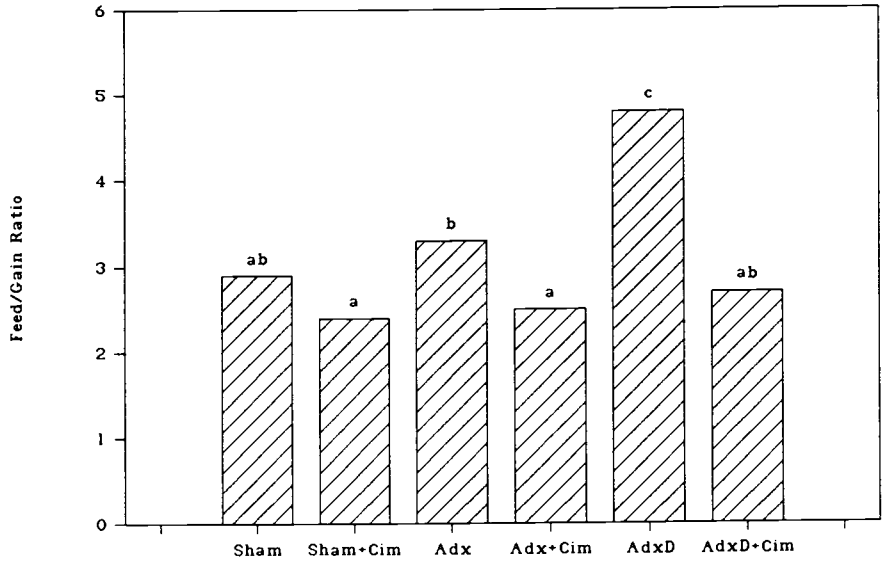


Figure 4 Effect of glucocorticoid status and cimaterol on feed efficiency.

Water intake was increased ($p < .05$) 2 fold by adrenalectomy (Adx) or adrenalectomy with dexamethasone treatment (AdxD) when compared to sham-controls (Sham). Cimaterol decreased ($p < .05$) water intake of Adx rats, but only slightly decreased ($p > .05$) the water intake of Sham and AdxD rats (Figure 5). Urine excretion was increased ($p < .05$) in Adx and AdxD rats when compared with Sham. In contrast, cimaterol decreased ($p < .05$) urine excretion of Adx and AdxD rats (Figure 6).

Effect of glucocorticoid status and cimaterol on tissue weights and tissue proportion are listed in Table 4. Upper hind-limb muscle weight (Figure 7) in control-fed animals were not affected by Adx, but were reduced ($p < .05$) by Dex treatment (AdxD). Lower hind-limb muscle weights (Figure 8), were not affected in Adx but were reduced ($p < .05$) in AdxD rats, compared to Sham. Cimaterol increased ($p < .05$) weights of the upper and lower hind-limb muscles on both Sham and AdxD rats, compared to their control-fed rats. Both hind-limb muscles of Adx animals were also slightly increased by cimaterol, however, the effects were not significant. When muscle weight was expressed as a proportion of final body weight (Figures 9 and 10), upper hind-limb muscle proportion was increased ($p < .05$) in Adx, but the lower hind-limb proportion was not affected. When Adx rats were given Dex treatment (AdxD), the proportion of upper hind-limb muscle was decreased ($p < .05$) compared to their Adx counterparts, but the proportion of lower hind-limb muscle was not affected. Cimaterol increased ($p < .05$) the proportion of lower hind-limb muscle irrespective of glucocorticoid status. However, proportions of upper hind-limb muscle

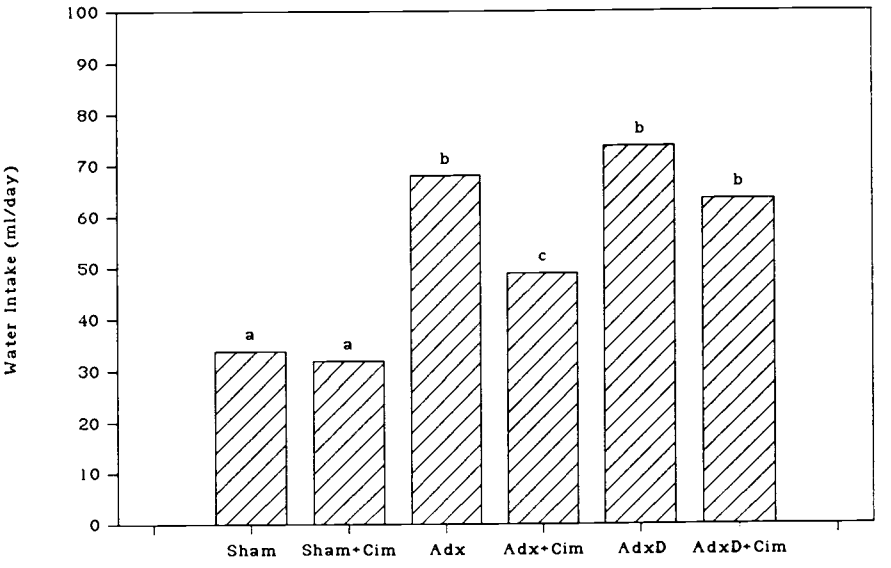


Figure 5 Effect of glucocorticoid status and cimaterol on average daily water intake.

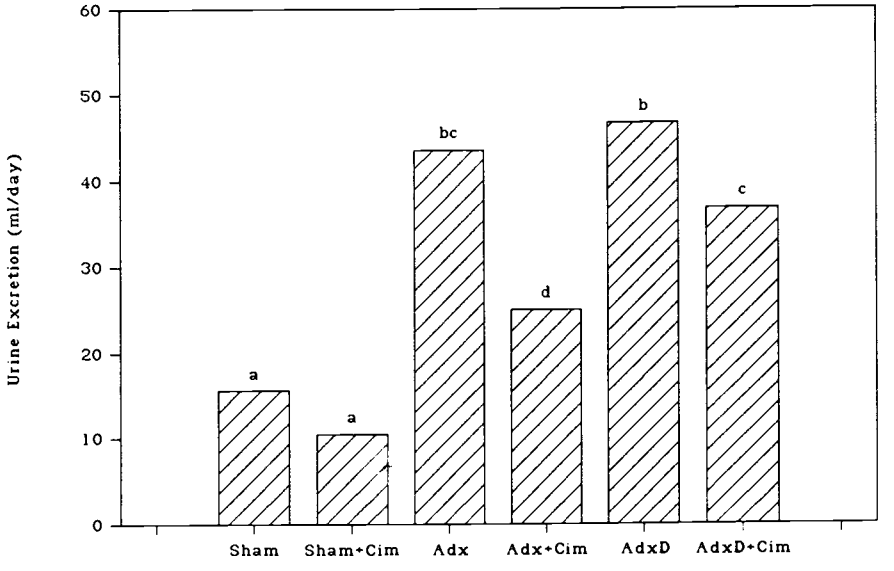


Figure 6 Effect of glucocorticoid status and cimaterol on average daily urine excretion.

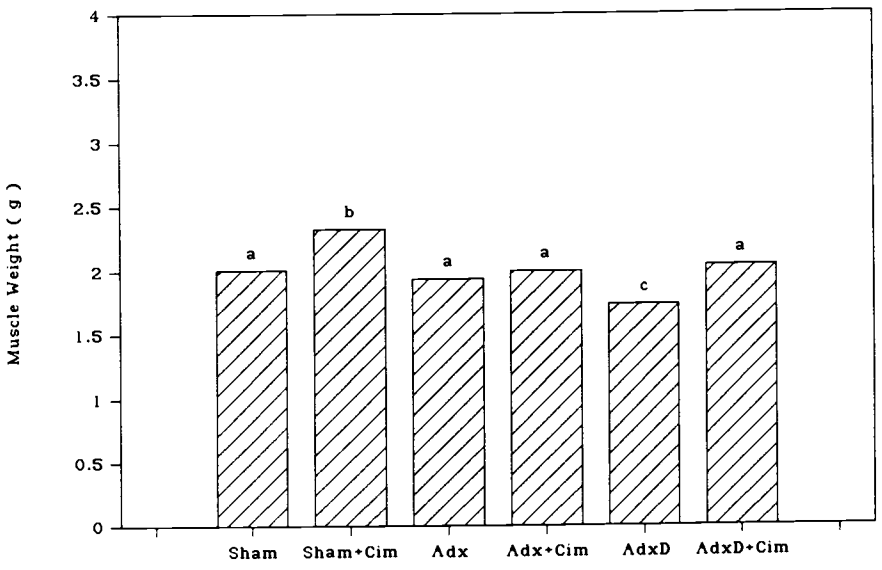


Figure 7 Effect of glucocorticoid status and cimaterol on left upper hind-limb muscle weight.

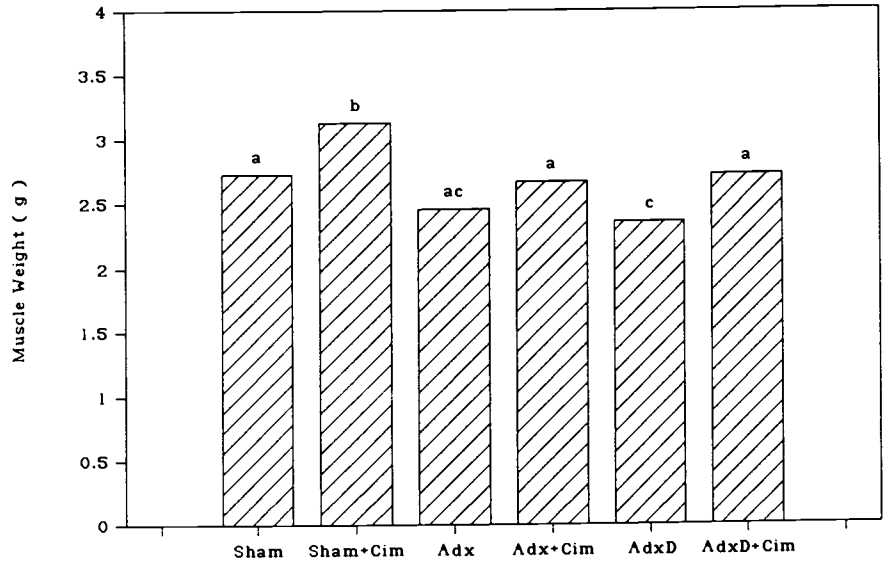


Figure 8 Effect of glucocorticoid status and cimaterol on left lower hind-limb muscle weight.

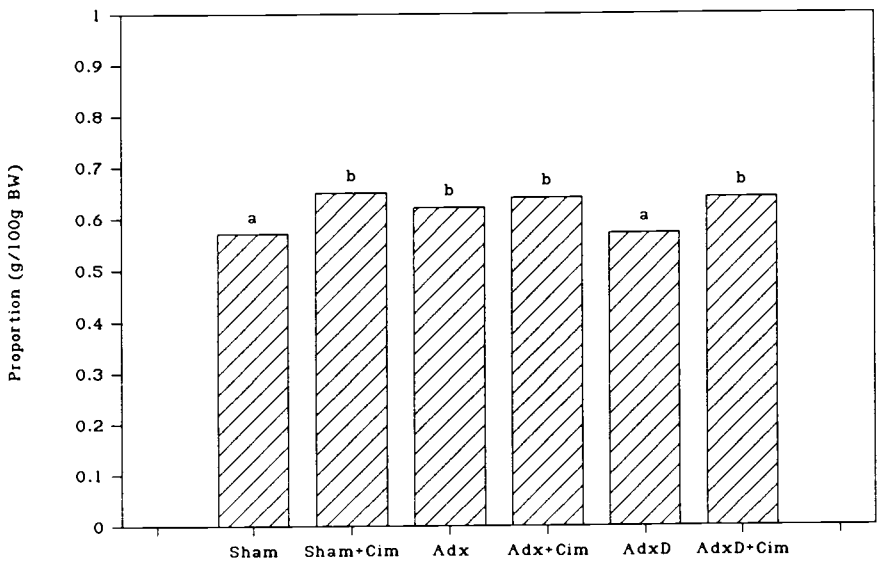


Figure 9 Effect of glucocorticoid status and cimaterol on left upper hind-limb muscle proportion.

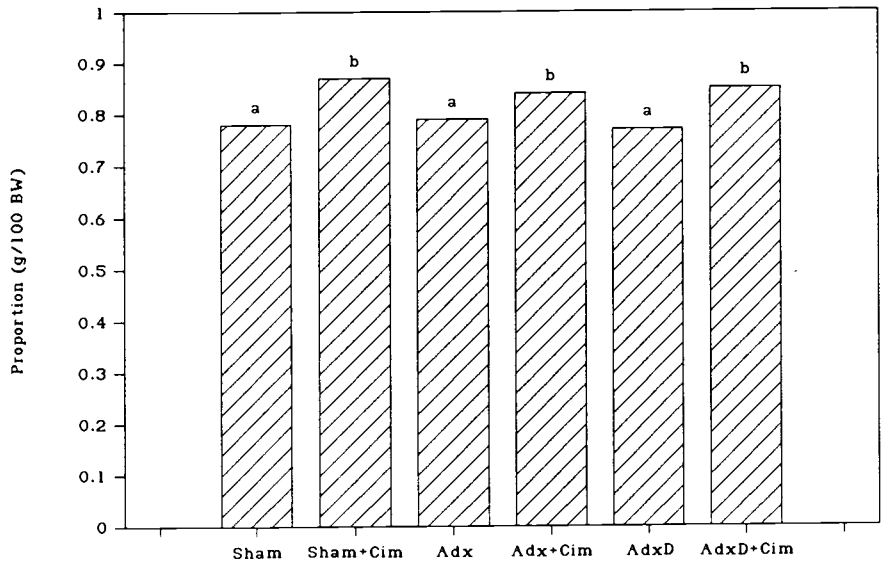


Figure 10 Effect of glucocorticoid status and cimaterol on left lower hind-limb muscle proportion.

Table 4. Effect of glucocorticoid status and cimaterol on tissue weights in rats[1].

	Expertimental Treatment					
	Sham	Sham+cim	Adx	Adx+cim	AdxD	AdxD+cim
Hind-limb muscle -Left upper (g)	2.01±.16^{a}	2.33±.14^{b}	1.94±.12^{a}	2.00±.19^{a}	1.74±.08^{c}	2.04±.09^{a}
-Left upper(g/100g BW)	.57±.04^{a}	.65±.04^{b}	.62±.04^{b}	.64±.02^{b}	.57±.02^{a}	.64±.02^{b}
-Left lower (g)	2.73±.18^{a}	3.13±.17^{b}	2.46±.25^{ac}	2.67±.29^{a}	2.36±.21^{c}	2.72±.25^{a}
-Left lower(g/100g BW)	.78±.04^{a}	.87±.04^{b}	.79±.05^{a}	.84±.03^{b}	.77±.05^{a}	.85±.06^{b}
Liver (g)	16.54±1.93^{a}	14.93±1.16^{ab}	13.22±1.49^{bc}	12.91±1.58^{bd}	15.33±1.39^{a}	14.52±1.68^{acd}
Liver (g/100g BW)	4.69±.43^{ab}	4.12±.31^{a}	4.22±.26^{a}	4.11±.49^{a}	4.99±.34^{b}	4.57±.53^{ab}
Heart (g)	.96±.04^{a}	1.05±.11^{a}	1.07±.06^{a}	.95±.09^{a}	.95±.08^{a}	1.00±.10^{a}
Heart (g/100g BW)	.28±.02^{a}	.30±.03^{a}	.33±.01^{a}	.30±.02^{a}	.31±.02^{a}	.31±.03^{a}
Kidney (g)	2.81±.16^{a}	2.56±.23^{ab}	2.62±.10^{ac}	2.15±.20^{bd}	2.48±.02^{ade}	2.32±.17^{bce}
Kidney (g/100g BW)	.80±.04^{ab}	.71±.05^{ac}	.83±.05^{b}	.68±.05^{cd}	.81±.01^{ab}	.72±.05^{abd}

[1] Values are mean±SE. Values in the same row with differing superscripts differ significantly ($p<.05$).

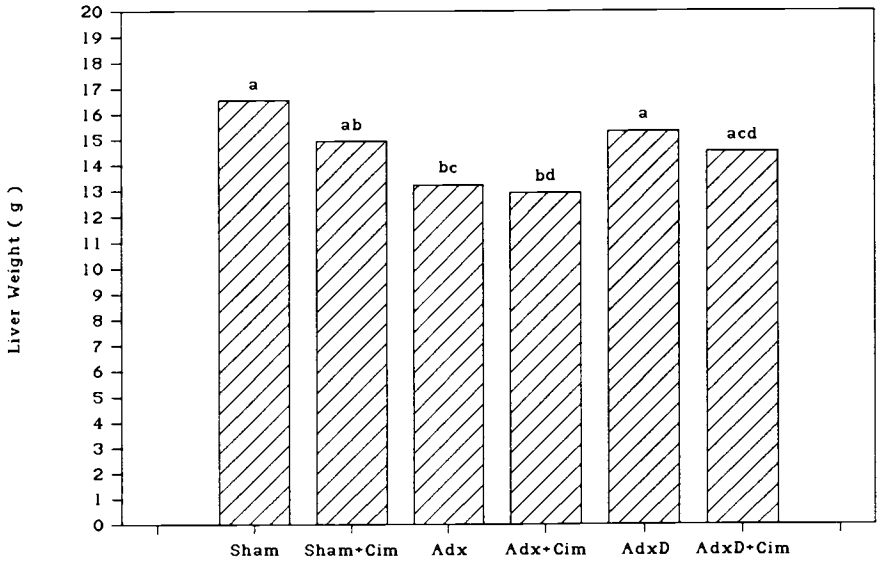


Figure 11 Effect of glucocorticoid status and cimaterol on liver weight.

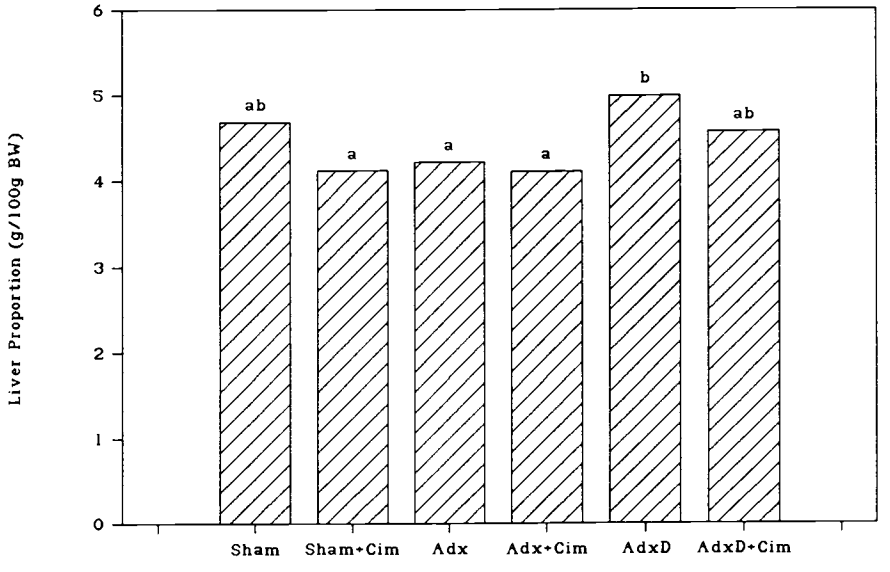


Figure 12 Effect of glucocorticoid status and cimaterol on liver proportion.

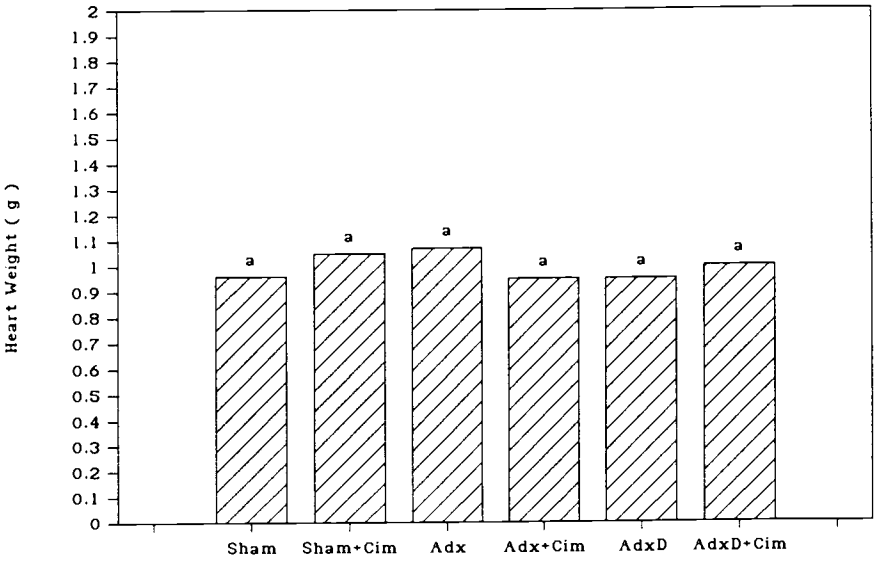


Figure 13 Effect of glucocorticoid status and cimaterol on heart weight.

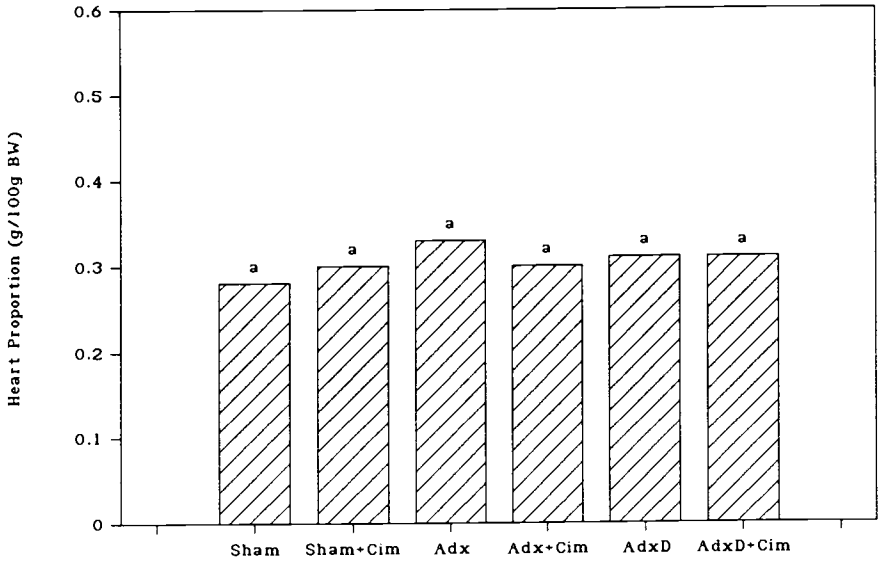


Figure 14 Effect of glucocorticoid status and cimaterol on heart proportion.

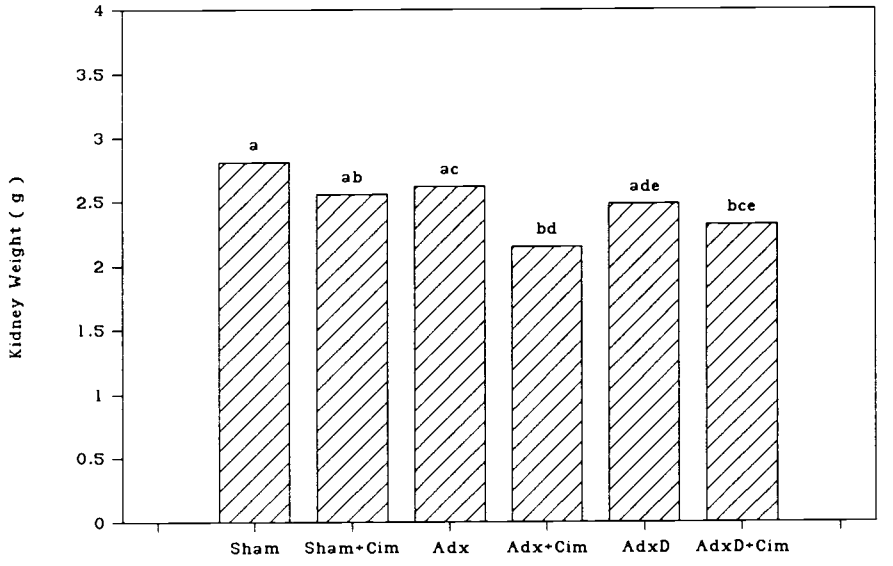


Figure 15 Effect of glucocorticoid status and cimaterol on kidney weight.

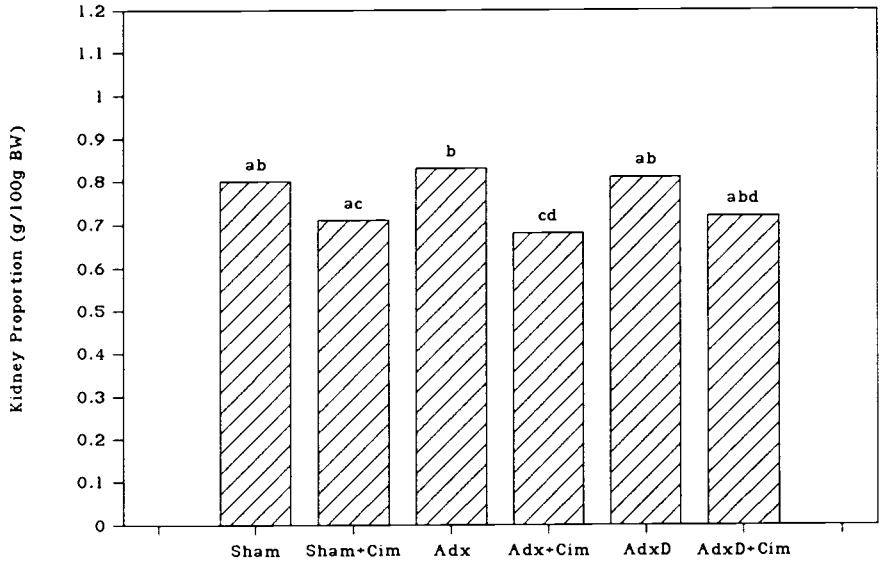


Figure 16 Effect of glucocorticoid status and cimaterol on kidney proportion.

were increased by cimaterol in Sham and AdxD but not in Adx animals, compared to their control-fed animals (Table 4).

Liver weights and liver as proportion of final body weight are shown in Figures 11 and 12. Liver weights were reduced ($p < .05$) by adrenalectomy. Dexamethasone treatment increased ($p < .05$) liver weights and liver proportion of adrenalectomized animals, compared to their Adx counterparts. The proportion of liver was not affected by cimaterol irrespective of glucocorticoid status (Table 4). Heart weight and heart as a proportion of final body weight were not affected by any of the treatments in this experiment (Figures 13 and 14). Kidney weight and kidney as a proportion of body weight were not affected by glucocorticoid status. Cimaterol decreased kidney weights irrespective of glucocorticoid status, however, only in Adx rats was this effect significant ($p < .05$). This was also true when kidney was expressed as a proportion of body weight.

Effect of glucocorticoid status and cimaterol on DNA, RNA and protein synthesis are listed in Table 5. Adrenalectomy increased ($p < .05$) DNA concentration in both muscle and liver when compared to sham-control; however, DNA concentration was decreased ($p < .05$) in both tissues by giving Dex treatment to Adx rats. Cimaterol decreased ($p < .05$) DNA concentration of Sham and Adx rats in muscle but not in liver. In contrast, cimaterol reduced ($p < .05$) DNA concentration of AdxD rats in liver but not in muscle (Figures 17 and 18).

Total DNA content in both muscle and liver (Figures 19 and 20) were not affected by adrenalectomy, but total muscle DNA content was reduced ($p < .05$) by Dex treatment when compared to their Adx counterparts. Cimaterol did not affect total DNA of Adx

rats in muscle or liver. Total DNA of Sham and AdxD rats in muscle was increased ($p < .05$) by cimaterol; however, total DNA of Sham and AdxD rats was reduced ($p < .05$) by cimaterol in liver. Muscle RNA concentration (Figure 21), an estimate of protein synthetic capacity (Reeds et al., 1986), was reduced ($p < .05$) in Adx rats and was increased ($p < .05$) in AdxD rats. Cimaterol increased ($p < .05$) muscle RNA concentration irrespective of glucocorticoid status. When total RNA content of muscle was considered (Figure 24), the same effects were observed as muscle RNA concentration.

Liver RNA concentration was increased ($p < .05$) by adrenalectomy and restored ($p < .05$) to levels similar to Sham by Dex treatment (Figure 28). Cimaterol increased ($p < .05$) liver RNA concentration of Sham and AdxD rats but not Adx rats. Total liver RNA content was not affected by adrenalectomy or cimaterol irrespective of glucocorticoid status (Figure 29). The ratio of RNA : DNA in muscle (Figure 25) was reduced by adrenalectomy and increased ($p < .05$) by Dex treatment. Cimaterol increased ($p < .05$) the RNA : DNA ratio in muscle irrespective of glucocorticoid status. Liver RNA : DNA ratio (Figure 30) was increased ($p < .05$) by cimaterol only in sham-controls.

Muscle protein synthesis was not affected in control-fed animals irrespective of glucocorticoid status. However, protein synthesis of cimaterol-fed animals was increased by adrenalectomy, compared to Sham animals.

Protein synthesis : RNA ratio (Figure 32), an estimate of protein synthetic effeciency

Table 5 . Effect of glucocorticoid status and cimaterol on DNA and RNA contents, RNA/DNA ratio and protein synthesis in rats[1].

	Exterimental Treatment					
	Sham	Sham+cim	Adx	Adx+cim	AdxD	AdxD+cim
Muscle						
DNA(mg/g tissue)	1.38±.06^{a}	1.32±.04^{b}	1.44±.04^{c}	1.33±.02^{b}	1.33±.04^{b}	1.33±.03^{b}
DNA(total g)	3.77±.30^{a}	4.14±.30^{b}	3.54±.29^{a}	3.54±.40^{a}	3.14±.31^{c}	3.60±.30^{a}
RNA(mg/g tissue)	1.06±.06^{a}	1.36±.09^{b}	.72±.06^{c}	1.16±.08^{d}	1.09±.07^{a}	1.43±.12^{e}
RNA(total g)	2.89±.36^{ab}	4.27±.34^{c}	1.79±.27^{d}	3.11±.47^{a}	2.57±.25^{b}	3.90±.51^{e}
RNA/DNA ratio	.77±.05^{a}	1.03±.09^{b}	.50±.05^{c}	.88±.06^{d}	.82±.07^{e}	1.08±.10^{b}
Protein synthesis (nmole Tyr/g/2hr)	2.08±.20^{a}	2.22±.29^{a}	2.70±.23^{ab}	3.08±.48^{b}	2.27±.25^{a}	2.44±.19^{ab}
P.S. efficiency (P.S./mg RNA/2hr)	1.91±.25^{ac}	1.72±.23^{a}	3.95±.29^{b}	2.88±.49^{c}	2.18±.34^{ac}	1.84±.15^{ac}
Liver						
DNA(mg/g tissue)	1.65±.08^{a}	1.55±.07^{a}	1.94±.09^{b}	1.97±.09^{b}	1.59±.13^{a}	1.41±.08^{c}
DNA(total g)	27.26±2.95^{a}	23.06±1.86^{b}	25.61±3.40^{ab}	25.54±4.00^{ab}	24.25±1.52^{ab}	20.38±2.35^{c}
RNA(mg/g tissue)	6.56±.29^{a}	7.76±.26^{b}	7.73±.33^{b}	7.92±.31^{b}	6.62±.56^{a}	6.21±.52^{c}
RNA(total g)	108.72±15.93^{a}	115.90±10.90^{a}	101.87±9.74^{ab}	102.25±13.13^{ab}	101.74±14.21^{ab}	90.23±12.95^{b}
RNA/DNA ratio	3.98±.27^{a}	5.03±.21^{b}	4.00±.23^{a}	4.02±.29^{a}	4.19±.44^{ac}	4.43±.41^{c}

[1] Values are mean±SE. Values in the same row with differing superscripts differ significantly ($p < .05$).

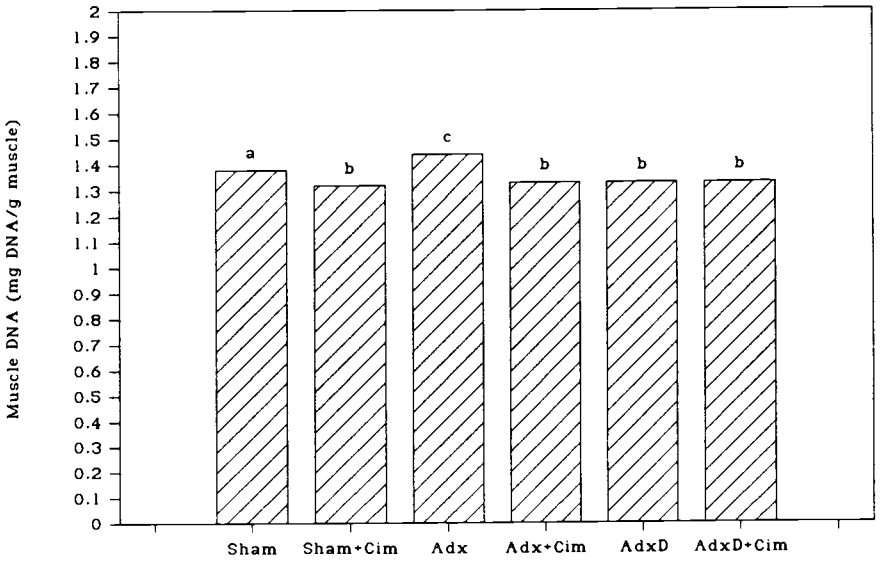


Figure 17 Effect of glucocorticoid status and cimaterol on DNA concentration of left lower hind-limb muscle.

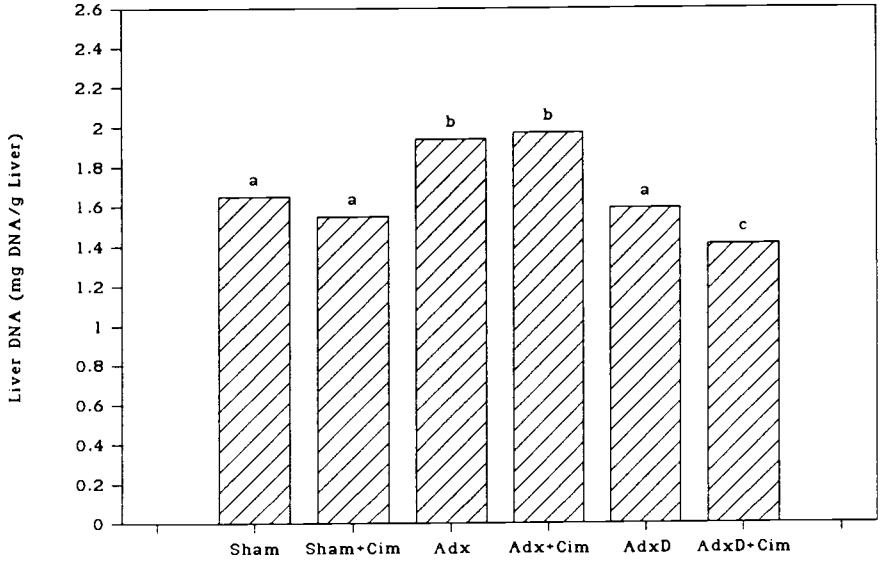


Figure 18 Effect of glucocorticoid status and cimaterol on DNA concentration of liver.

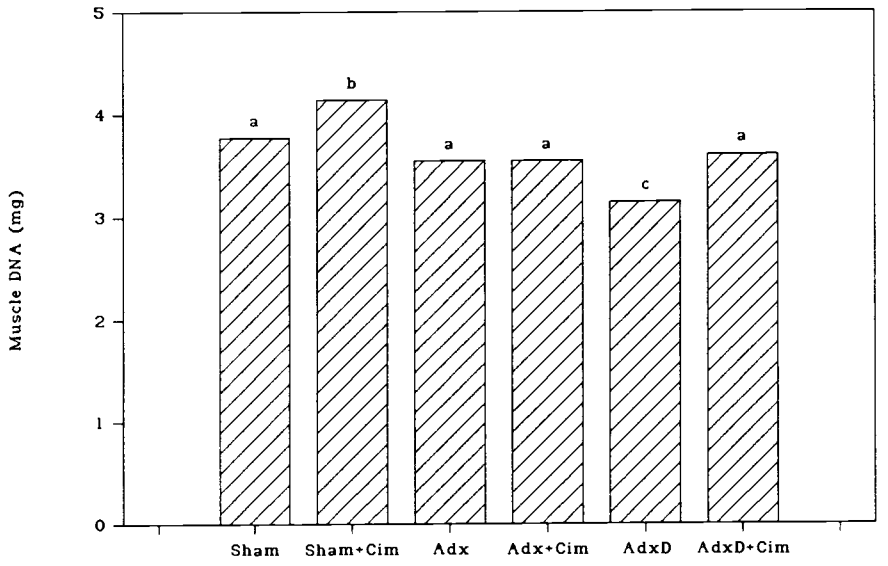


Figure 19 Effect of glucocorticoid status and cimaterol on total DNA content of left lower hind-limb muscle.

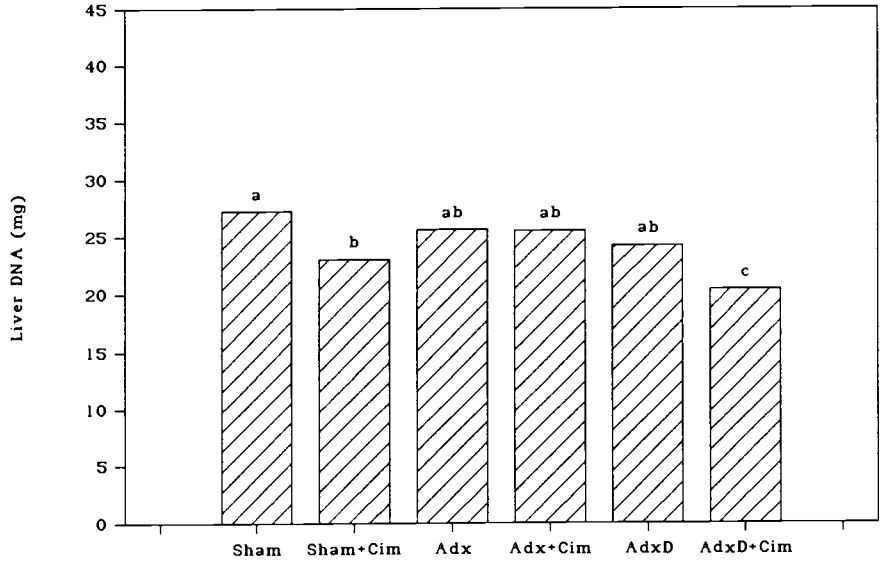


Figure 20 Effect of glucocorticoid status and cimaterol on total DNA content of liver.

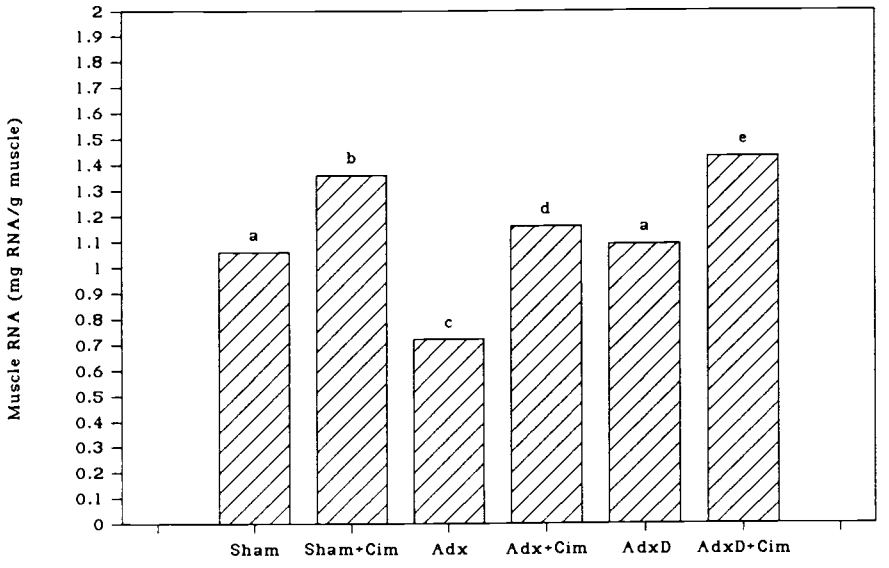


Figure 21 Effect of glucocorticoid status and cimaterol on RNA concentration of left lower hind-limb muscle.

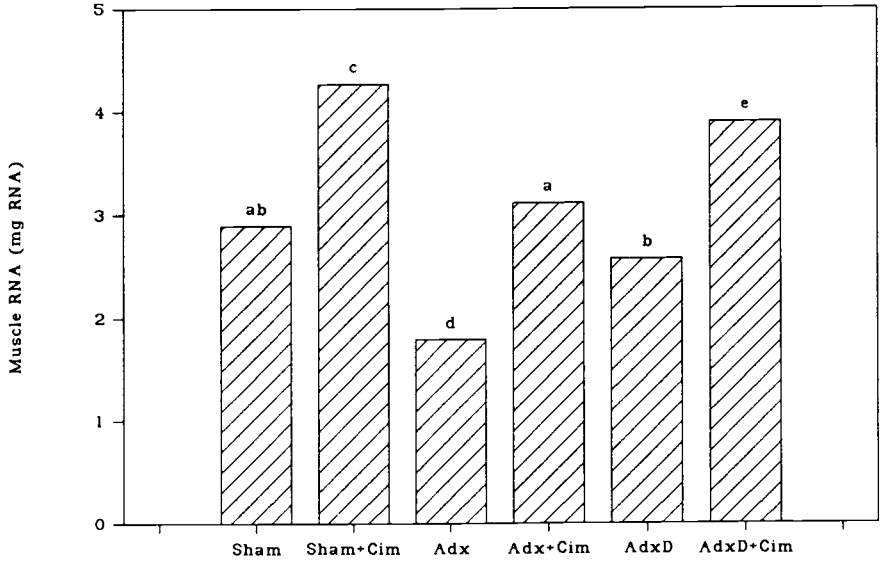


Figure 22 Effect of glucocorticoid status and cimaterol on total RNA content of left lower hind-limb muscle.

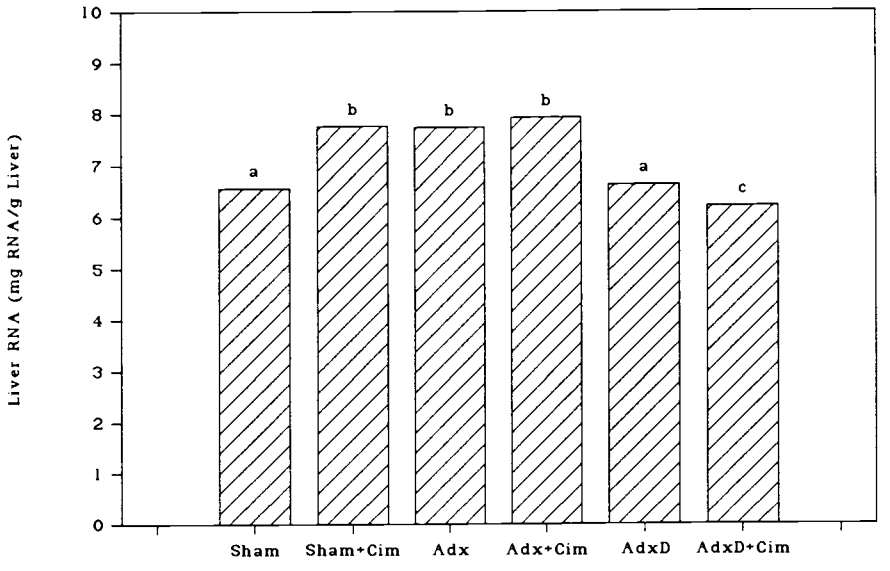


Figure 23 Effect of glucocorticoid status and cimaterol on RNA concentration of liver.

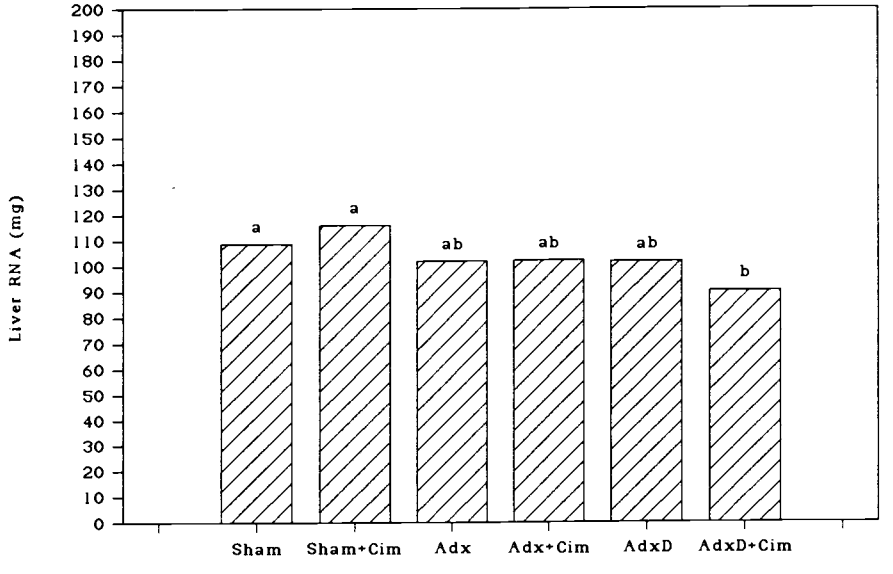


Figure 24 Effect of glucocorticoid status and cimaterol on total RNA content of liver.

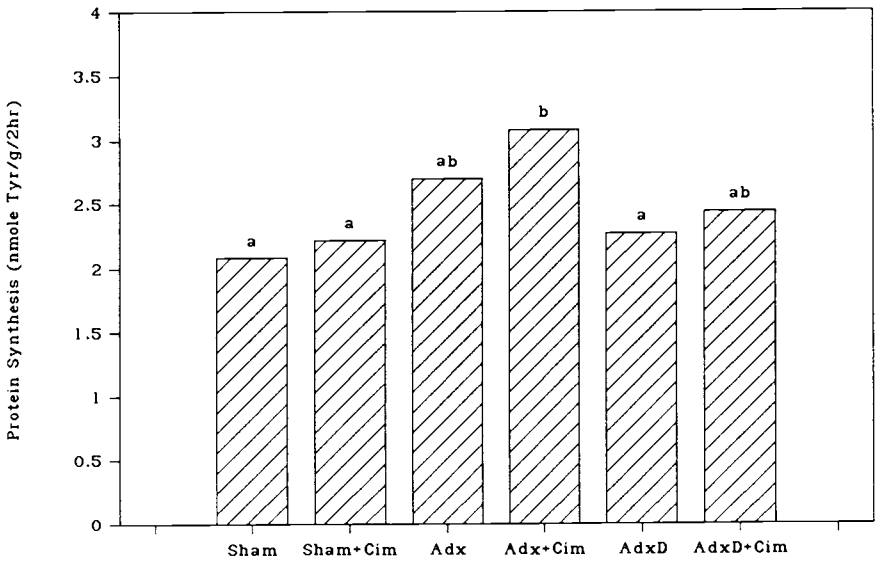


Figure 25 Effect of glucocorticoid status and cimaterol on in vitro protein synthesis of isolated epitrochlearis muscle.

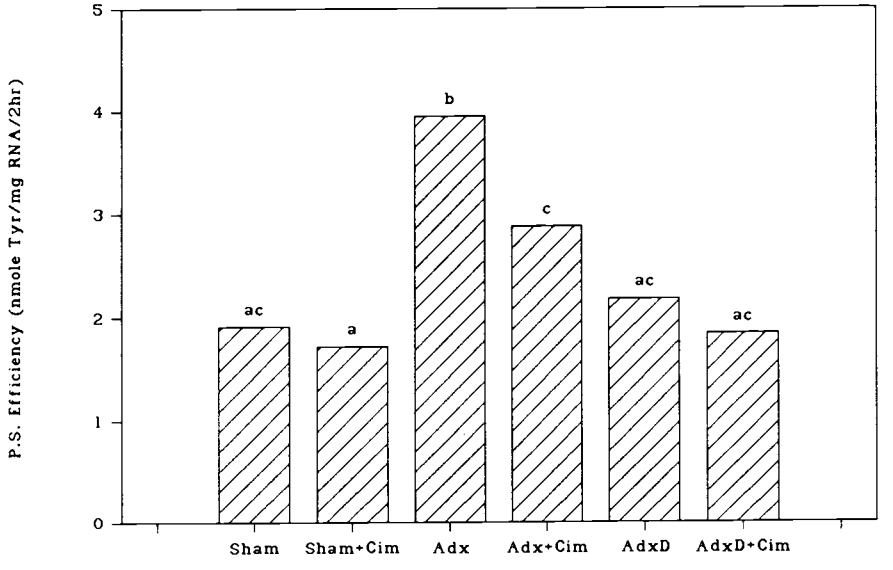


Figure 26 Effect of glucocorticoid status and cimaterol on in vitro protein synthitic efficiency of isolated epitrochlearis muscle.

(Reeds et al., 1986), was increased ($p < .05$) by adrenalectomy and was decreased ($p < .05$) to near-sham-control levels by giving Dex treatment to Adx rats. Cimaterol tend to decrease protein synthesis : RNA ratio irrespective of glucocorticoid status, however only in Adx rats was this effect significant ($p < .05$).

Effect of glucocorticoid status and cimaterol on urinary NMH excretion are listed in Table 6. Urinary NMH excretion of Adx and AdxD rats was increased ($p < .05$) throughout the study when compared to sham-controls. Dexamethasone treatments increased ($p < .05$) urinary NMH excretion on Day 2 but reduced ($p < .05$) it on Day 7 compared to their Adx counterparts. Cimaterol did not affect urinary NMH excretion of sham-controls throughout the study, but decreased ($p < .05$) urinary NMH excretion of Adx rats on Day 1, Day 2 and Day 7. The urinary NMH excretion of AdxD rats was not affected by cimaterol on Day 1 and Day 7 but was decreased ($p < .05$) on Day 2 and Day 4, compared to their Adx counterparts. Urinary NMH excretion of sham-controls was increased to 3 folds on Day 4 and 2 folds on Day 7 when compared with NMH excretion of Day 1. Similar effects were observed in cimaterol-fed sham-controls and Dex-treated control-fed animals. Urinary NMH excretion of Adx rats was increased over time and reached highest level on Day 7 (Figures 27 - 34).

Effects of glucocorticoid status and cimaterol on several proteolytic enzyme activities are shown on Table 7. Adrenalectomy or treatment with Dex did not affect the cathepsin B activity in muscle and liver (Figures 35 and 36). Cimaterol increased ($p < .05$) cathepsin B activities in muscle irrespective of glucocorticoid status; however, cimaterol

Table 6. Effects of glucocorticoid status and cimaterol on the urinary NMH excretion in rats[1].

	Experimental Treatment					
	Sham	Sham+cim	Adx	Adx+cim	AdxD	AdxD+cim
Urinary NMH						
Day 1						
(μm/100g BW/day)	.10±.03[a]	.21±.07[a]	2.74±.87[b]	1.69±.69[c]	2.44±.70[b]	2.63±.66[b]
(μm/rat/day)	.30±.10[a]	.65±.23[a]	7.49±2.61[d]	4.56±1.82[c]	6.45±1.86[d]	6.73±1.72[d]
Day 2						
(μm/100g BW/day)	.10±.04[a]	.30±.14[a]	3.05±1.09[b]	2.27±.47[c]	4.43±1.27[d]	2.15±.21[c]
(μm/rat/day)	.31±.11[a]	.94±.44[a]	8.56±3.21[b]	6.12±1.27[c]	12.12±3.11[d]	5.73±.62[c]
Day 4						
(μm/100g BW/day)	.31±.10[a]	.32±.12[a]	3.41±.53[bd]	2.91±.50[cd]	3.29±.50[bd]	2.51±.56[c]
(μm/rat/day)	.99±.32[a]	1.05±.39[a]	9.99±1.61[b]	8.18±1.14[c]	9.50±1.35[b]	7.40±1.73[c]
Day 7						
(μm/100g BW/day)	.22±.07[a]	.13±.04[a]	3.76±.50[b]	2.36±.51[c]	2.57±.35[c]	2.31±.56[c]
(μm/rat/day)	.78±.26[a]	.46±.16[a]	11.70±1.15[b]	7.48±1.90[b]	7.89±.90[c]	7.37±1.84[c]

[1] Values are mean±SE. Values in the same row with differing superscripts differ significantly ($p < .05$).

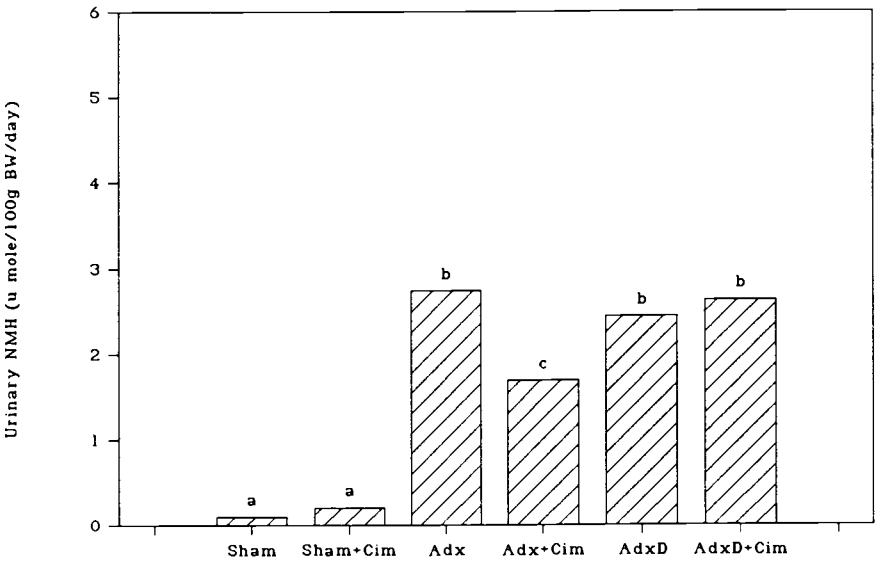


Figure 27 Effect of glucocorticoid status and cimaterol on day 1 urinary NMH excretion.

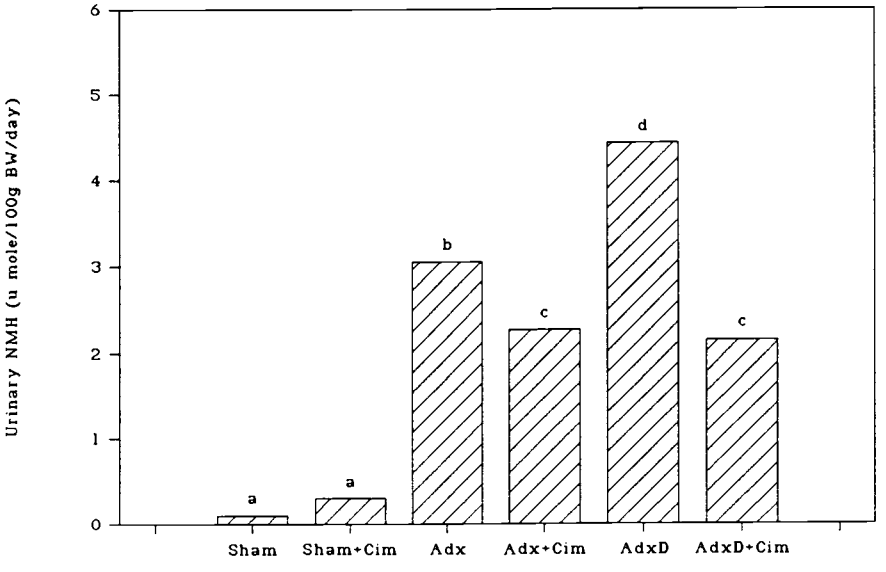


Figure 28 Effect of glucocorticoid status and cimaterol on day 2 urinary NMH excretion.

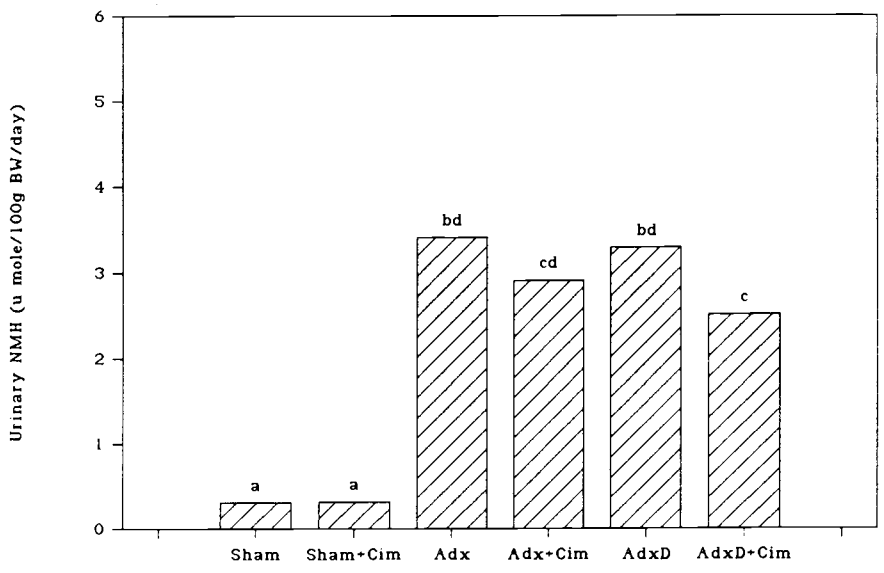


Figure 29 Effect of glucocorticoid status and cimaterol on day 4 urinary NMH excretion.

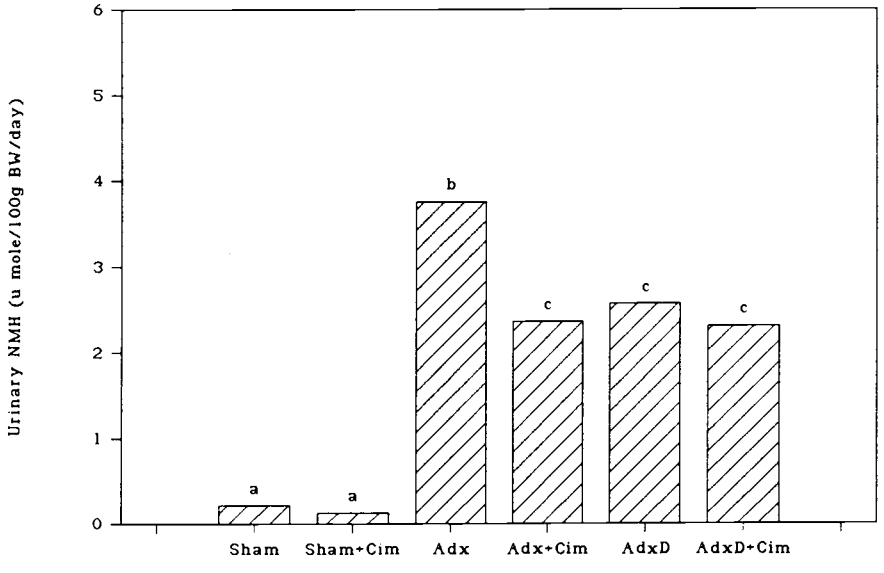


Figure 30 Effect of glucocorticoid status and cimaterol on day 7 urinary NMH excretion.

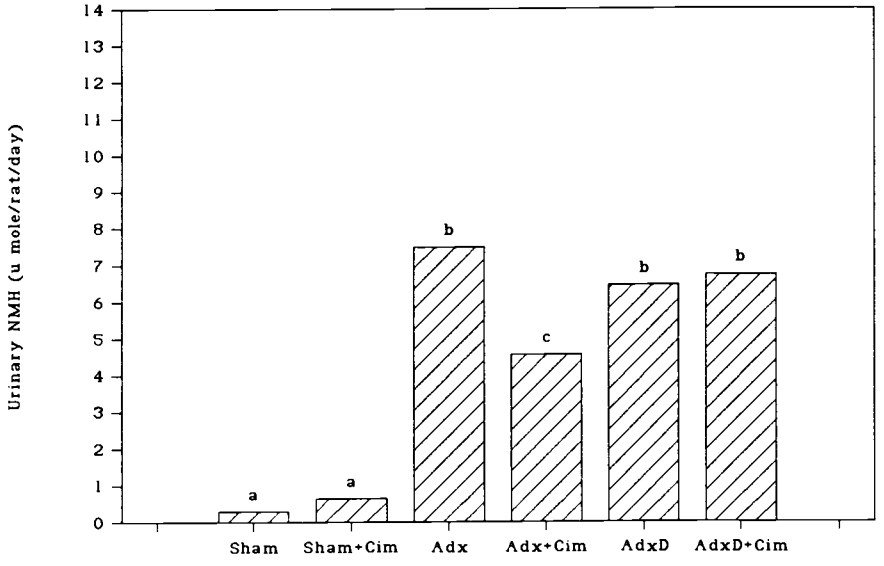


Figure 31 Effect of glucocorticoid status and cimaterol on day 1 urinary NMH excretion.

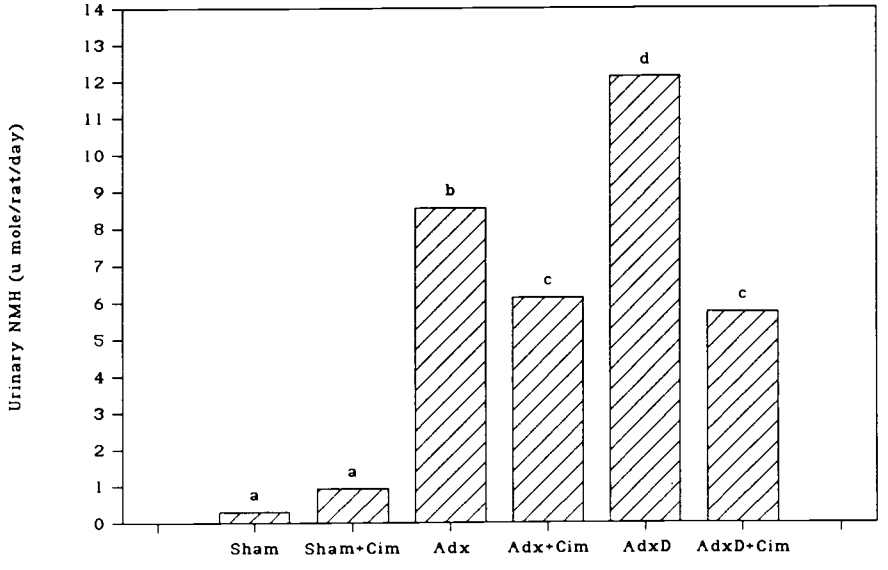


Figure 32 Effect of glucocorticoid status and cimaterol on day 2 urinary NMH excretion.

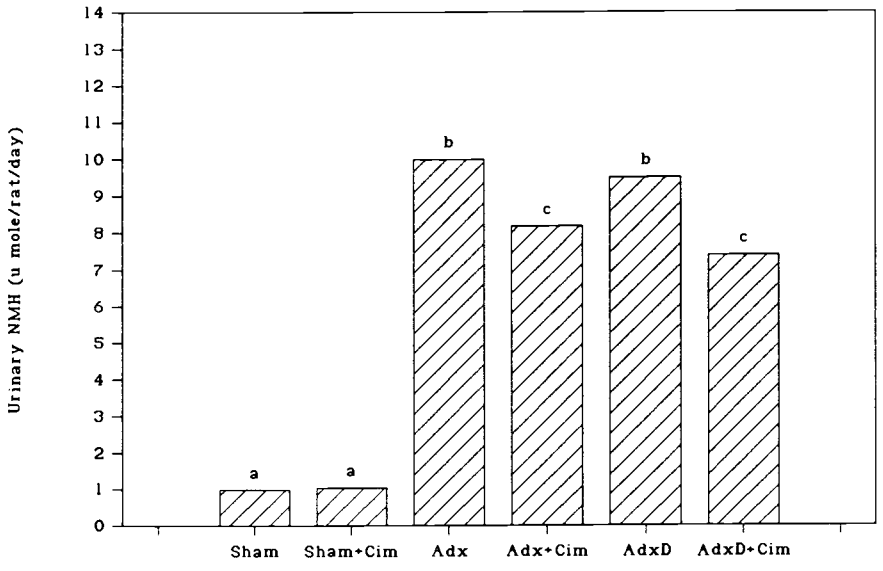


Figure 33 Effect of glucocorticoid status and cimaterol on day 4 urinary NMH excretion.

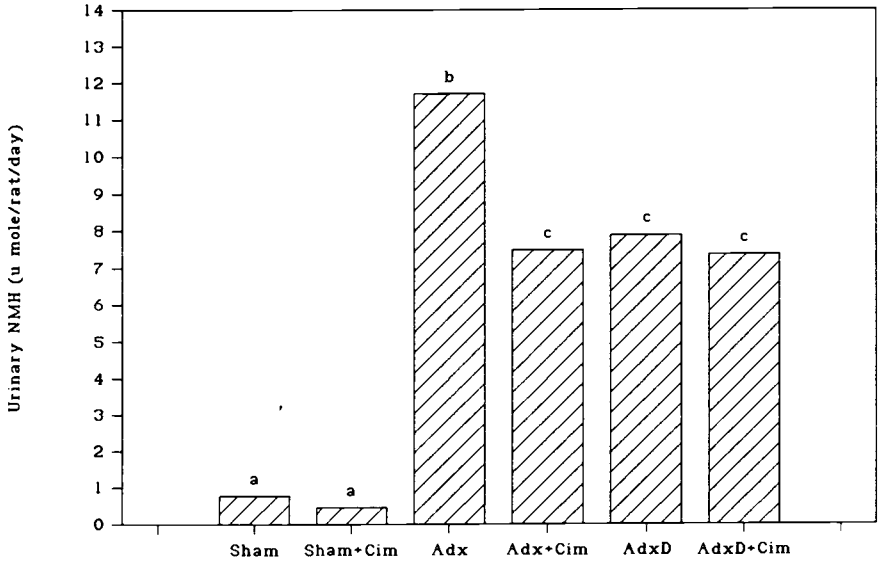


Figure 34 Effect of glucocorticoid status and cimaterol on day 7 urinary NMH excretion.

decreased ($p < .05$) cathepsin B activities of sham-controls in liver. Cathepsin B activity of liver was about seven- to eleven-fold higher than that detected in muscle.

Cathepsin D activity in muscle (Figure 37) was reduced ($p < .05$) by adrenalectomy and restored by Dex treatment to near Sham level. Adrenalectomy or dexamethasone treatment did not affect the cathepsin Dactivity in liver (Figure 38) of control-fed animals. Cimaterol increased ($p < .05$) cathepsin D activities of Adx animals in muscle; however, it decreased ($p < .05$) the cathepsin D activity of AdxD rats in both muscle and liver. Liver also had 2.5-3.5 fold higher cathepsin D activity than muscle (Table 7).

Muscle cathepsin L activities (Figure 39) of control-fed animals were increased ($p < .05$) by adrenalectomy and decreased ($p < .05$) by treatment of dexamethasone. Cimaterol increased ($p < .05$) muscle cathepsin L activities of Sham and AdxD rats but did not affect muscle cathepsin L activities of Adx animals when compared to control-fed animals. Liver cathepsin L activity (Figure 40) was not affected in this study. However, cathepsin L activity in liver is about two-fold higher than in muscle.

Neutral protease activity was increased ($p < .05$) by adrenalectomy in both muscle and liver (Figures 41 and 42), Dexamethasone treatment decreased ($p < .05$) the neutral protease activity in liver compared to its Adx counterparts. Cimaterol decreased ($p < .05$) neutral protease activity in muscle irrespective of glucocorticoid status; however it increased the neutral protease activities of Adx rats in liver, compared to control-fed animals. Neutral protease activity in muscle was 3- to 8-fold higher than in liver (Table 7).

Table 7. Effects of glucocorticoid status and cimaterol on activities of several proteolytic enzymes in muscle and liver of rats[1].

	Experimental Treatment					
	Sham	Sham+cim	Adx	Adx+cim	AdxD	AdxD+cim
Muscle						
Cathepsin D	8.11±1.05[a]	8.03±.53[a]	5.44±.94[b]	9.01±.93[a]	8.02±.79[a]	6.72±1.03[c]
Cathepsin L	6.84±.45[a]	7.70±.36[b]	7.59±.33[b]	7.49±.65[b]	6.37±.39[c]	6.91±.63[a]
Cathepsin B	1.32±.07[a]	1.48±.06[b]	1.31±.05[a]	1.80±.06[c]	1.24±.09[a]	1.45±.07[b]
Neutral proteinase	.51±.03[a]	.37±.05[b]	.59±.05[c]	.45±.04[d]	.57±.08[c]	.41±.03[b]
mCDP	37.18±3.82[a]	31.86±3.05[b]	34.21±1.54[b]	43.54±3.50[c]	32.84±1.99[b]	37.99±3.68[a]
μCDP	17.57±2.39[a]	12.43±2.34[b]	11.77±1.95[b]	18.60±2.79[a]	10.28±1.83[b]	12.52±1.35[b]
Calpastatin	122.97±15.5[a]	168.20±19.7[b]	146.40±24.7[a]	221.11±23.9[c]	125.40±18.4[a]	190.31±21.1[d]
Liver						
Cathepsin D	23.49±3.01[a]	21.24±1.92[a]	21.94±2.86[a]	24.76±2.91[a]	23.48±2.35[a]	18.14±2.88[b]
Cathepsin L	12.48±1.38[a]	12.43±1.89[a]	12.97±1.56[a]	13.35±1.63[a]	14.14±1.08[a]	14.09±1.83[a]
Cathepsin B	16.79±.62[a]	14.26±1.33[b]	15.99±2.42[ab]	15.29±.97[ab]	14.63±1.39[b]	16.06±1.15[ab]
Neutral proteinase	.10±.01[a]	.09±.01[a]	.12±.01[ab]	.16±.02[b]	.06±.01[c]	.06±.01[c]

[1] Values are mean±SE. Values in the same row with differing superscripts differ significantly ($p < .05$).

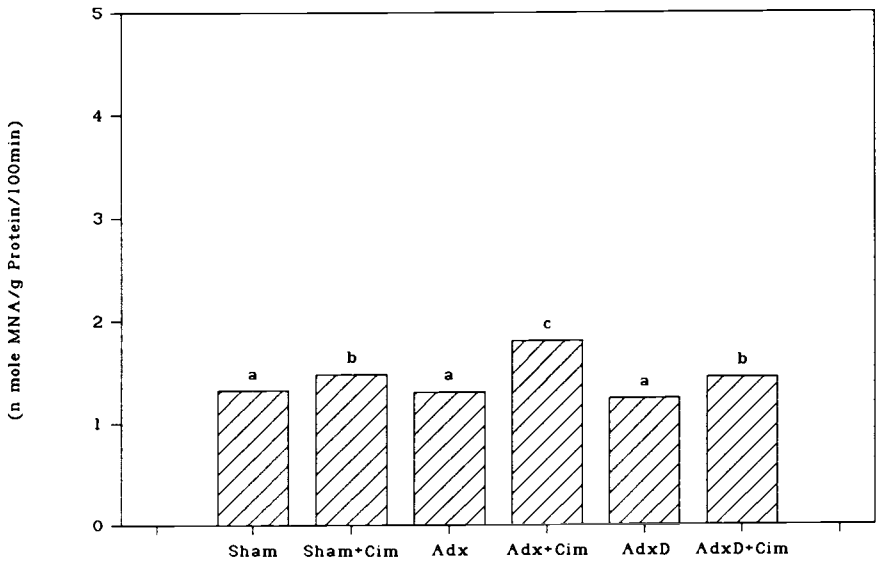


Figure 35 Effect of glucocorticoid status and cimaterol on muscle cathepsin B activity.

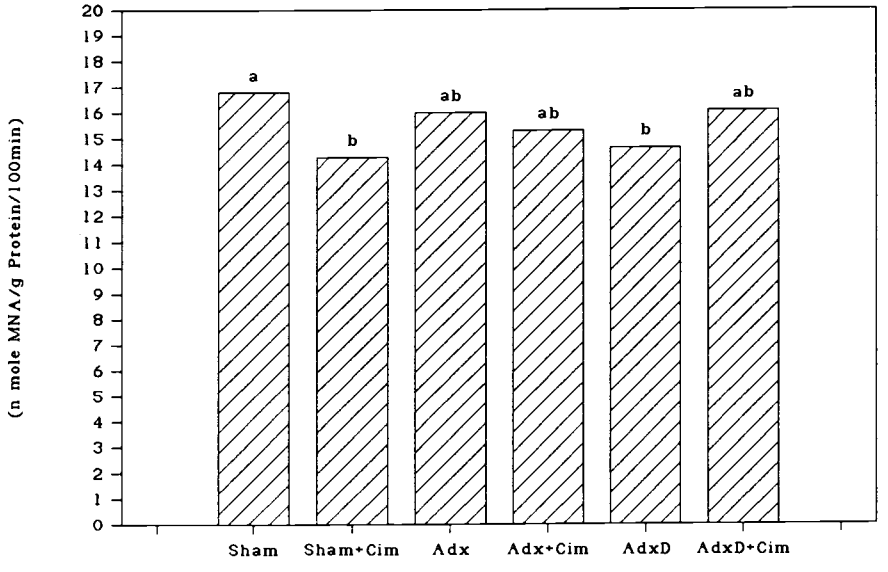


Figure 36 Effect of glucocorticoid status and cimaterol on liver cathepsin B activity.

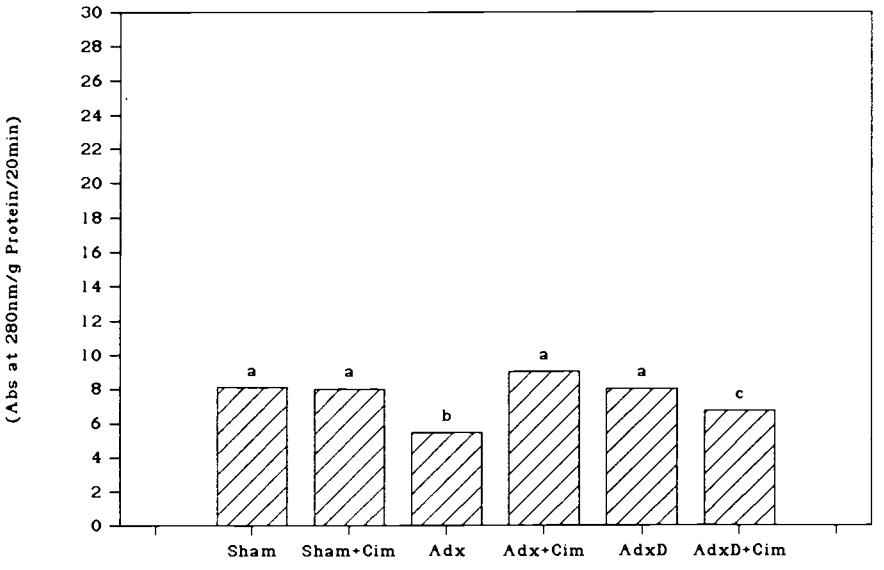


Figure 37 Effect of glucocorticoid status and cimaterol on muscle cathepsin D activity.

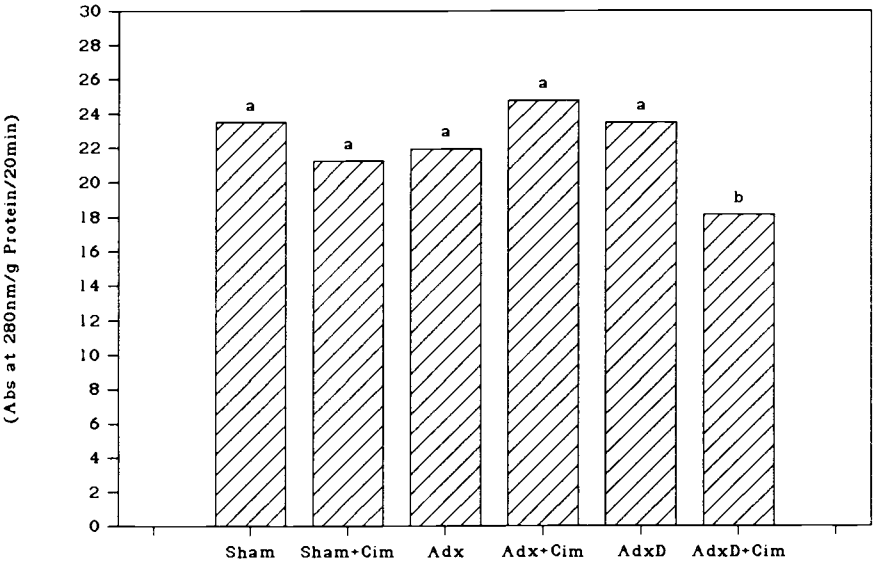


Figure 38 Effect of glucocorticoid status and cimaterol on liver cathepsin D activity.

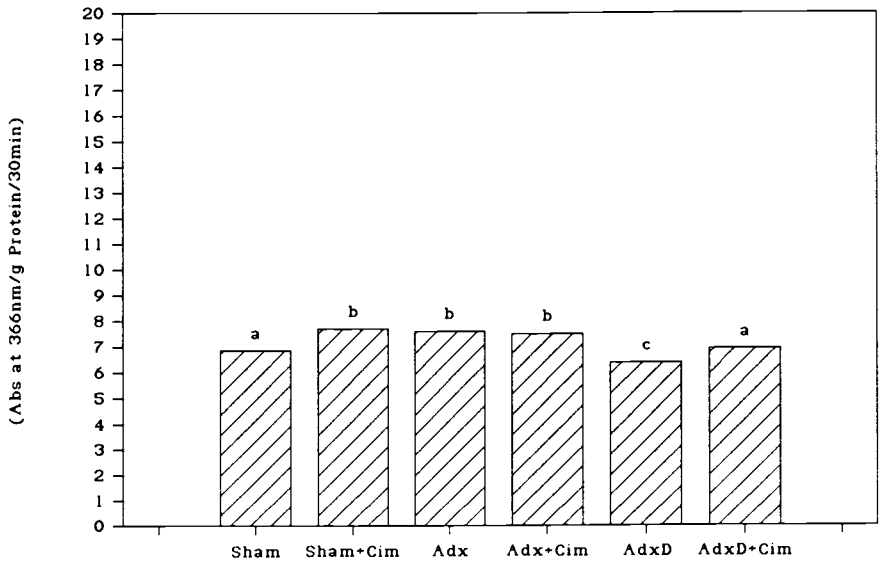


Figure 39 Effect of glucocorticoid status and cimaterol on muscle cathepsin L activity.

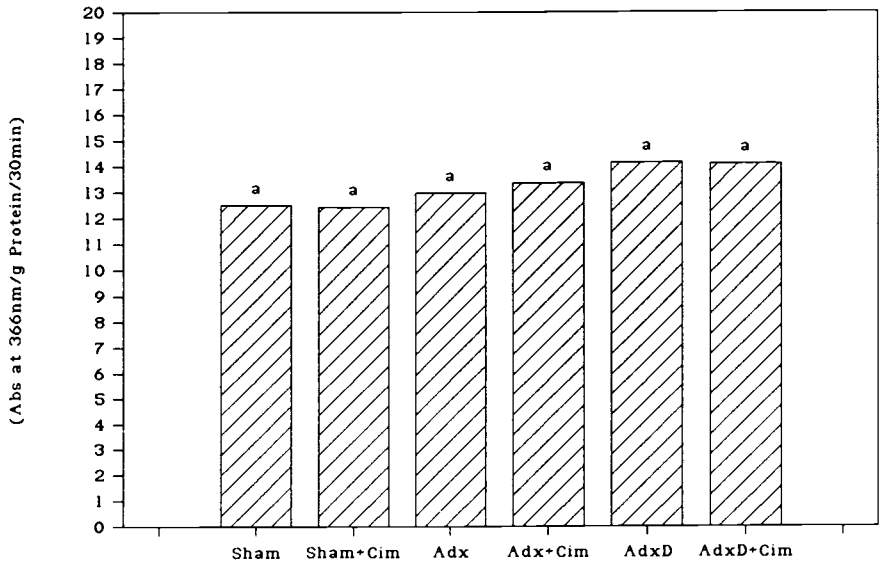


Figure 40 Effect of glucocorticoid status and cimaterol on liver cathepsin L activity.

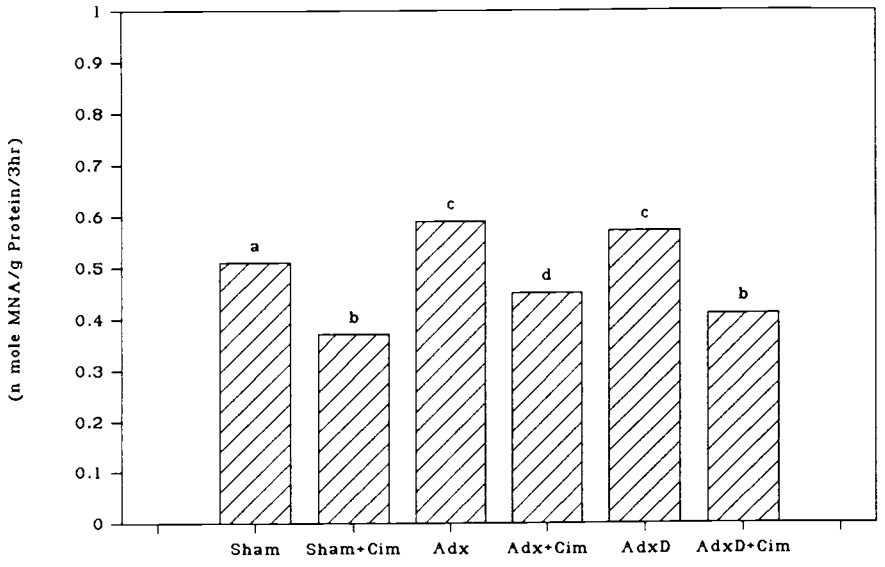


Figure 41 Effect of glucocorticoid status and cimaterol on muscle neutral proteinase activity.

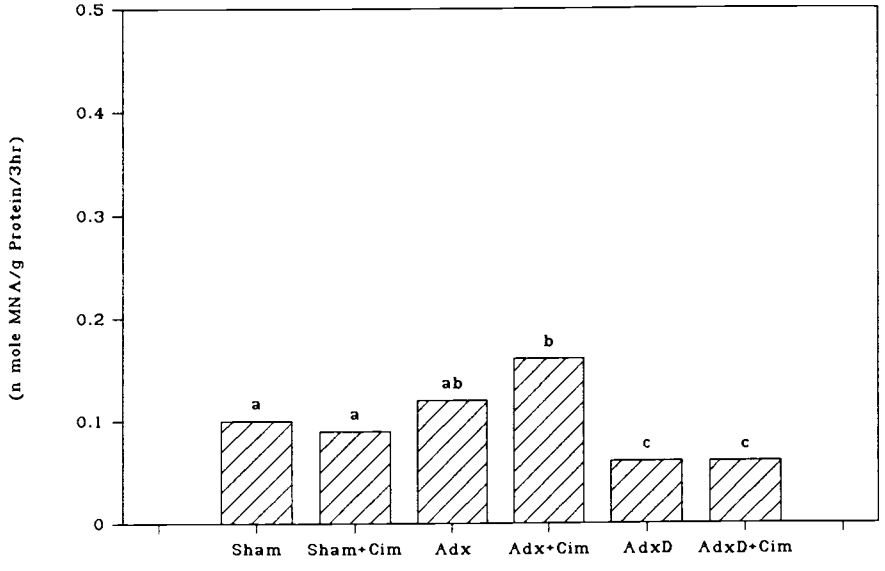


Figure 42 Effect of glucocorticoid status and cimaterol on liver neutral proteinase activity.

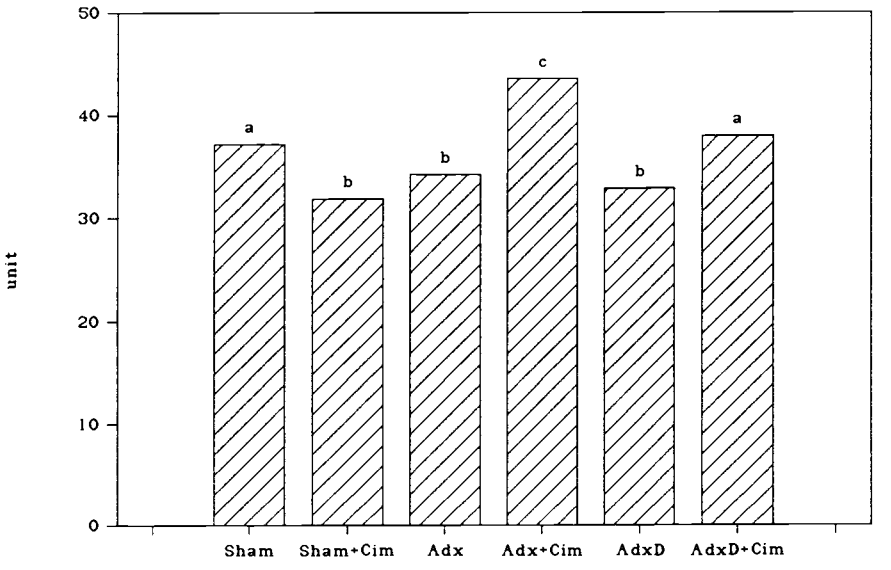


Figure 43 Effect of glucocorticoid status and cimaterol on muscle mCDP activity.

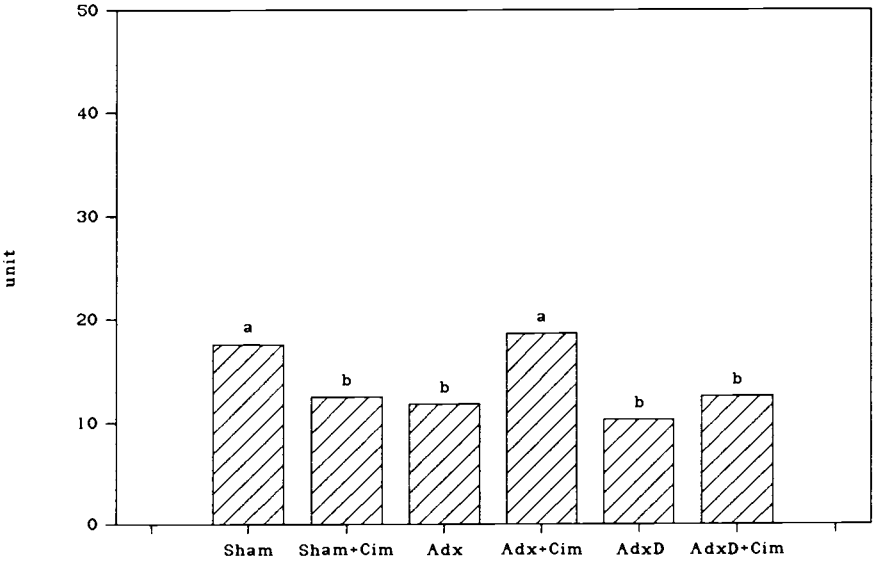


Figure 44 Effect of glucocorticoid status and cimaterol on muscle μCDP activity.

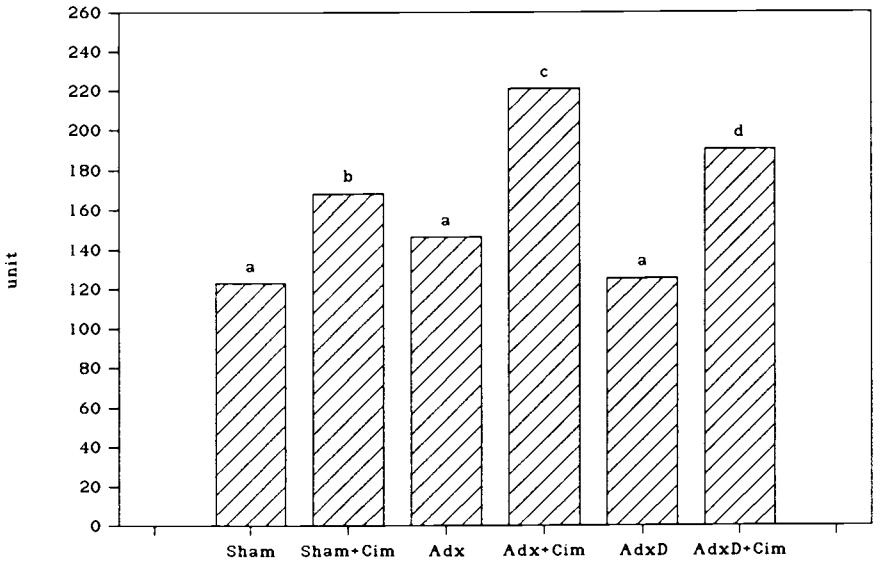


Figure 45 Effect of glucocorticoid status and cimaterol on muscle calpastatin activity.

Both adrenalectomy and dexamethasone treatments reduced ($p < .05$) mCDP and μCDP activities (Figures 43 and 44) to the same levels in muscle of control-fed animals when compared to sham-controls. Cimaterol reduced ($p < .05$) both mCDP and μCDP activities of sham-controls. However, cimaterol increased ($p < .05$) both mCDP and μCDP activites of Adx animals, and increased ($p < .05$) mCDP activities of AdxD animals when compared to control-fed animals. In cimaterol-fed animals, activities of both mCDP and μCDP were increased ($p < .05$) by adrenalectomy compared to sham-controls, and reduced ($p < .05$) by Dex treatment when compared to its Adx counterparts (Table 7).

Activity of calpastatin (Figure 45) was not affected by glucocorticoid status in control-fed animals. Cimaterol increased ($p < .05$) activities of calpastatin in sham-control, Adx and AdxD animals by 37 %, 51 % and 52 %, respectively. In cimaterol-fed animals, activity of calpastatin was increased ($p < .05$) by Adx compared to sham-controls and reduced ($p < .05$) by Dex treatment compared to its Adx counterparts.

DISCUSSION

EFFECTS OF GLUCOCORTICOID STATUS ON MUSCLE

Adrenalectomy caused a reduction in average daily gain (ADG) which resulted from reduced feed intake rather than from a change in efficiency of gain. Dexamethasone treatment further reduced ADG which was caused by a reduction in efficiency of gain. Feed intake in dexamethasone-treated rats was increased when compared to their adrenalectomized counterparts and equivalent to sham-controls.

In previous studies (May et al., 1986), injection of 1.2 μg/100 g/day of dexamethasone was reported to be a physiological replacement dose in rats. Sharpe et al.(1986) found that a dose of 2.5 μg/100 g BW/day of dexamethasone when administered in the diet to 200 g rats partially restored growth ($p > .05$) or at least was not catabolic. Based on their studies we selected our dexamethasone dose; however, dexamethasone at this level in our study was a catabolic dose. This was indicated by reduced gain of animals in the treatment and by reduced muscle weights.

Short-term alterations in glucocorticoid status caused only minor changes in muscle as a proportion of body weight. In control-fed animals left upper hind-limb muscle, expressed as a proportion of body weight, was increased slightly by adrenalectomy and reduced with dexamethasone treatment. Left lower hind-limb muscle, expressed as a proportion of body weight, was not affected by glucocorticoid status. Reasons for differences in response to treatment are uncertain; however, may be due to muscle fiber

types associated with muscle samples. Odedra et al., 1983) reported that glycolytic plantaris or gastrocnemius muscle appeared to be more susceptible to the effect of the glucocorticoids (corticosterone) than was the more oxidative soleus muscle and it is possible that left upper hind-limb muscle consisted of a higher proportion of glycolytic muscle fiber.

To study a mechanism by which glucocorticoids may affect muscle weights we examined effects of treatments on urinary N^{τ}-methylhistidine (NMH) excretion on days 1, 2, 4 and 7 of the study and also examined protein synthesis in isolated epitrochlearis muscle bundles. NMH excretion have been used as an index of myofibrillar protein degradation (Young and Munro, 1978).

Adrenalectomy and dexamethasone treatment did not affect protein synthesis; however, protein synthetic efficiency (Protein synthesis/RNA) and protein synthetic capacity (RNA concentration) were affected by glucocorticoid status. Glucocorticoids appear to maintain RNA concentration because adrenalectomy reduced RNA concentration and because dexamethasone treatment restored RNA concentration when expressed as RNA/g tissue, as total RNA/muscle or as RNA/mg DNA; however, the effects of glucocorticoids on protein synthetic efficiency were opposite. Adrenalectomy increased protein synthetic efficiency in muscle and dexamethasone treatment reduced it. Hence, glucocorticoids appeared not to exert changes in protein synthesis on day 8 of the study in control-fed animals and we have no evidence that the reduction in muscle weight in control-fed Dex-treated rats was due to a reduction in protein synthesis.

However, adrenalectomy of cimaterol-fed animals caused a significant increase in

protein synthesis which resulted from a large increase in protein synthetic efficiency and was accompanied by small decrease in synthetic capacity. This implies that under some circumstances glucocorticoids may repress protein synthesis. The ability of glucocorticoids to reduce protein synthesis has been reported previously (Rannels and Jefferson, 1980; Odedra et al., 1983). This change in protein synthesis on day 8 of the study was associated with a decrease in muscle weights. Enhanced muscle protein degradation may have accounted for reduced muscle weight in adrenalectomized and Dex-treated groups.

Both adrenalectomy and Dex treatment resulted in several-fold increases in urinary NMH excretion on days 1, 2, 4 and 7 when compared to sham-control. Similars effects of adrenalectomy on urinary NMH excretion have been reported (Lowell et al.,1986); however, in this study adrenalectomy resulted in only a 2 fold increase in NMH. Reasons for differences between our study and this study are not known.

Several investigations (Tomas et al., 1979; Santidrian et al., 1981; Odedra et al., 1983; Kayali et al., 1987) have shown that glucocorticoid-treatment increases urinary NMH excretion and our data support these studies. Tomas et al. (1979) reported that glucocorticoids will temporally regulate urinary NMH excretion and again, our data are supportive. In control-fed animals, urinary NMH excretion was increased by Dex treatment on day 1, 2, 4 and 7 of the study; however, the greatest effect was detected on day 2 after which NMH excretion decreased. Similar patterns of urinary NMH excretion were not observed in Dex treated cimaterol-fed animals. The implication of the latter observation will be discussed later.

The mechanism by which muscle weights are reduced in Dex-treated animals compared to sham-controls may involve control of myofibrillar protein degradation. Muscle weights of Dex-treated animals were smaller than sham-controls and Dex-treatment enhanced urinary NMH excretion throughout the study.

The mechanism by which muscle weights are reduced in Dex-treated animals compared to adrenalectomized animals is unclear. Protein synthesis was reduced by Dex-treatment; however, this effect was not significant. Urinary NMH excretion was increased on Day 2 by Dex treatment, when compared to adrenalectomized animals, but on day 7 Dex-treated animals excrete less NMH than their adrenalectomized counterparts. There are two possible explanations for this. First, protein synthesis may have been reduced by Dex treatment earlier in the study, when assessment of this parameter was not possible. Alternatively, changes in urinary NMH excretion may have resulted from changes in degradation of the gut NMH pool rather than from changes in the skeletal muscle NMH pool. Although the gut NMH pool accounts for only a small percentage of total body NMH (Wassner and Li, 1982), large changes in the rate of degradation could bias estimates of myofibrillar protein degradation with this technique.

Twice daily injection of vehicle may have caused stress to our animals. This was indicated by increased excretion of NMH in sham-control animals. On days 4 or 7, urinary NMH excretion was increased compared to days 1 and 2 and implies possible glucocorticoid-dependent regulation of muscle protein degradation.

To elucidate a mechanism by which changes in urinary NMH excretion may be mediated, we examined activities of several proteolytic enzymes which have been

implicated in degradation of muscle proteins. These included calcium-dependent proteinases, which are believed to initiated degradation of myofibrillar proteins in vivo (Dayton et al., 1976), neutral proteinase, which has been implicated in pathology associated with several human myopathic disorders (Kar and Pearson, 1980), and three lysosomal cathepsins. In is unlikely that lysosomal cathepsins play important roles in regulation of myofibrillar protein degradation; however, they have been implicated in sarcoplasmic protein degradation (Bird et al., 1980; Goldspink and Lewis, 1985). Recent evidence supports the belief that lysosomes are involved in degradation of muscle proteins (Gerard et al., 1988) although this study did not identify which proteins are substrates for lysosomal proteolysis.

Adrenalectomy and Dex treatment resulted in large increases in NMH excretion compared to sham-controls. Cathepsin L activity was increased slightly by adrenalectomy and reduced by Dex-treatment thus resembling the pattern of urinary NMH excretion caused by variable glucocorticoid status. However, the magnitude of changes in cathepsin L activity did not approach the magnitude of changes in urinary NMH excretion on day 7 control by the same treatments. Neutral proteinase activity was increased by adrenalectomy but not reduced by Dex treatment. Cathepsin D activity was opposite the response of cathepsin L. Avtivities of both mCDP and μCDP were reduced by adrenalectomy and Dex treatment did not further reduce activities of mCDP and μCDP which implies that changes in activities of muscle CDPs were not responsible for the enhanced urinary NMH excretion in Adx and AdxD treatments.

If changes in urinary NMH excretion identified in this study have arised solely from

changes in degradation of muscle protein it is unlikely that alterations in muscle cathepsin L and neutral proteinase could account for the magnitude of change in NMH. This indicates that other muscle proteinases could be responsible for changes in urinary NMH excretion we detected. Alternatively targeting of myofibrillar proteins for degradation could represent the regulated event in their degradation.

The opposite effects of glucocorticoid status on cathepsin D versis cathepsin L are interesting and imply coordinate yet opposing regulation and also imply that these enzymes are involved in different processes.

EFFECTS OF GLUCOCORTICOIDS AND ITS INTERACTIONS WITH CIMATEROL ON MUSCLE

Cimaterol stimulated growth (ADG) of sham-operated and Dex-treated animals but not of adrenalectomized animals. It is interesting that cimaterol could only exert its anabolic effects in the presence of glucocorticoids. A similar effect was observed on muscle weight; cimaterol only stimulated left upper and lower parts of hind-limb muscle weights in sham-operated and Dex-treated animals.

Sharpe et al.(1986) reported that anabolic effects of a β-agonist on total gain and muscle weight in rats were glucocorticoid-dependent. Their study was conducted by using a replacement dose of Dex therapy and clenbuterol. In their study Dex treatment did not significantly improve growth although there was a tendency for growth to be improved. In our study, Dex treatment further reduced growth when compared to adrenalectomized

rats. In the study of Sharpe et al.(1986) clenbuterol increase growth of both sham-operated and Dex-treated rats but not in adrenalectomized rats. Based on this, the authors concluded that glucocorticoids exert permissive effects for the anabolic actions of β-agonists.

Sillence et al.(1985) reported that physiological levels of glucocorticoids inhibit growth. It is generally held that glucocorticoids are "catabolic" hormones. An alternative interpretation of the above data would be that clenbuterol antagonizes the growth-inhibiting properties of glucocorticoids.

If glucocorticoids exert permissive effects on the actions of cimaterol, we would expect that cimaterol-fed animals receiving Dex treatment would gain faster than cimaterol-fed adrenalectomized animals. Since this was not observed, we propose that the ability of cimaterol to enhance growth in sham-operated and Dex-treated rats relates to its ability to antagonize the growth-inhibiting actions of glucocorticoids.

Others have reported that glucocorticoids inhibit growth (Sillence et al.,1985). Treatment of female rats with trilostane cause a 20 % reduction in plasma corticosterone and was accompanied by a 22 % increase in growth. In our study adrenalectomy resulted in a reduction in growth which may have been partly due to an associated reduction in feed intake. It also implies that glucocorticoids at low concentrations are required for normal growth but higher concentration are catabolic.

The effects of cimaterol in urinary NMH excretion on days 1, 2, 4 and 7 of the study as well as protein synthesis in isolated epitrochlearis muscle bundles were again used to study the mechanisms by which cimaterol may stimulate growth and muscle

weights.

Dietary cimaterol (25 ppm) slightly increased protein synthesis irrespective of glucocorticoid status on day 8 of the study, although, none of these effects were significant. The mechanisms by which cimaterol and other β-adrenergic agonists stimulate muscle protein accretion in vivo have been examined previously. Emery et al.(1984) reported that clenbuterol increased muscle protein synthesis in growing rats and Helferich et al.(1988) reported that ractopamine increased muscle alpha-actin biosynthesis in swine. Other investigators (Reeds et al., 1986; Bohorov et al., 1987), however, found no effects of clenbuterol on muscle protein synthesis in rats and sheep, respectively, and concluded that β-agonist-dependent muscle growth promotion was mediated by a reduction in muscle protein degradation. The reasons for discrepencies among these studies are not known.

Protein synthetic capacity (RNA concentration) in muscle was increased by cimaterol irrespective of glucocorticoid status when expressed as RNA/g tissue, as total RNA/muscle or as RNA/DNA. However, effects of cimaterol on protein synthetic efficiency were different; cimaterol decreased protein synthetic efficiency in muscle, although, only in adrenalectomized animals was this effect significant. Hence, cimaterol appeared not to exert an effect on muscle protein synthesis on day 8 of the study. We therefore have no evidence that the different effects of cimaterol on weight gain and muscle weight in different glucocorticoid status were due to changes in protein synthesis.

The effects of glucocorticoids and cimaterol on protein synthetic efficiency raise two possible explanations for the interaction in regulation of this parameter. Cimaterol was able to reduce protein synthetic efficiency (PSE) only in the absence of glucocorticoids.

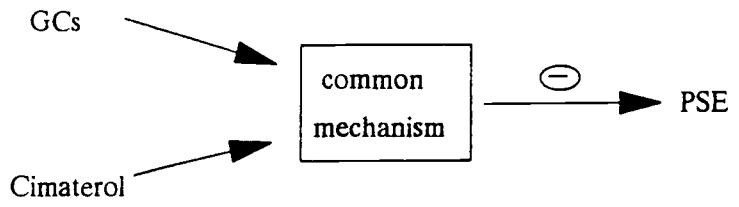
This implies either cimaterol and glucocorticoids reduce PSE via a common mechanism (Model 1) and, in this case, the effect of cimaterol is not additive or evident in sham-operated or Dex-treated rats or that glucocorticoids and cimaterol reduce PSE through different mechanisms and also antagonize each other on their ability to reduce PSE (Model 2).

Since cimaterol increased muscle RNA capacity irrespective of glucocorticoid status it appears the ability of cimaterol to regulate muscle RNA capacity is glucocorticoid-independent. In the absence of glucocorticoids, however, cimaterol tended to have a greater effect on RNA concentration than it did in the presence of glucocorticoids. In the absence of cimaterol adrenalectomy tended to cause a greater reduction in RNA capacity than it did in the presence of cimaterol.

Earlier we speculated that cimaterol enhanced growth by antagonizing growth-inhibiting properties of glucocorticoids. Evidence of such an antagonism was not evident in our protein synthesis data and was not evident when we examined the effects of cimaterol and glucocorticoids on protein synthetic capacity and efficiency. Hence, we have no evidence that an antagonism between glucocorticoids and cimaterol is manifest in control of protein synthesis. This implies that the antagonism, if it exists, may be directed towards control of protein degradation or was manifest in control of protein synthesis earlier in the study but was not detected on day 8 of our study.

The data on urinary NMH excretion assist in explaining effects of glucocorticoids and cimaterol on growth and muscle weights. The reduction in upper hind-limb muscle weights caused by adrenalectomy was associated with increased urinary NMH excretion.

Model 1



Model 2

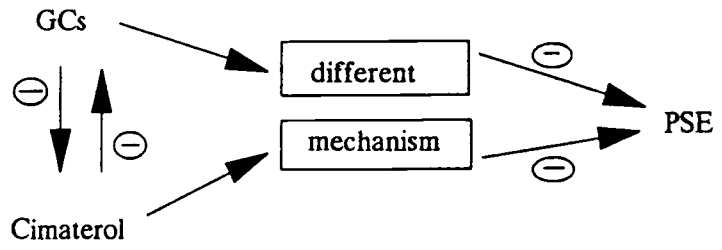


Figure 46 Proposed models by which glucocorticoid and cimaterol regulate protein synthetic efficiency.

The reductions in weights of both upper and lower hind-limb muscles of Dex-treated rats were associated with increased urinary NMH excretion. Therefore, reduced muscle weight caused by adrenalectomy and Dex-treatment is likely mediated by accelerated myofibrillar protein degradation. Similar results have been published. Lowell et al.(1986) reported that adrenalectomy enhanced urinary NMH by 2-fold and several authors (Tomas et al., 1979; Santidrian et al., 1981; Odedra et al., 1983; Kayali et al., 1987) have reported that catabolic doses of glucocorticoids temporally enhance urinary NMH excretion or released NMH from perfused hind-limb quarter by several-fold i.e. glucocorticoids initially increase NMH release (2-4 days of treatment) but continued

glucocorticoid treatment is associated with restoration of basal levels of NMH.

Examination of temporal control of urinary NMH excretion in our study yielded similar results. In Dex-treated rats, urinary NMH excretion was increased following 1 day of Dex treatment, further increased on day 2 of Dex treatment and thereafter declined. By day 7 of the study, urinary NMH excretion had fallen to a level similar to day 1 of Dex-treatment. These levels, however, remained several-fold higher than basal-levels. In other studies (Tomas et al., 1979; Odedra et al., 1983; Kayali et al., 1987), by day 8 of glucocorticoid treatment near-basal levels of urinary NMH excretion had been attained following a several-fold increase in NMH excretion. Reasons for the differences between our study and previous studies are uncertain. It is possible that if the study had been continued for several more days, basal levels of urinary NMH excretion may have been restored. Also, basal rates of urinary NMH excretion in previous studies were approximately 1 μmole/100g BW/day whereas basal rates in our rats were much lower (.22 μmole/100g BW/day). Stimulated rates in our study and in previous studies were similar. Our low basal NMH excretion maybe due to the age and size of our rats. We used approximately 300 g rats whereas Tomas et al. (1979) used 120 g rats. It is well known that excretion of NMH, as index of myofibrillar protein degradation, declines as animals age (Siebrits and Barnes, 1989). Hence, a longer period of time may have been required for our rats to re-establish basal NMH excretion patterns.

The ability of cimaterol to antagonize glucocorticoids was evident in Dex-treated rats on days 2 and 4. In these rats Dex treatment resulted in increased NMH excretion, which may account for decreased muscle weight and cimaterol antagonized this effect by

lowering urinary NMH excretion. Because cimaterol did not reduce urinary NMH on days 1 and 7 of the study it appears that it antagonizes only the large dex-dependent increased in urinary NMH. The abilities of cimaterol to improve growth of Dex-treated rats therefore may be due to its ability to antagonize Dex-dependent enhancement of myofibrillar protein degradation.

Cimaterol also reduced the adrenalectomy-dependent increase in urinary NMH excretion on days 1, 2 and 7. Although these effects were accompanied by a slight increase ($p > .05$) in protein synthesis, these changes did not result in a significant increase in muscle weight.

It is unclear why our estimates of protein synthesis and degradation do not reflect the inability of cimaterol to change muscle weight. We observed increased protein synthesis in epitrochlearis in cimaterol-fed adrenalectomized animals; however, it is possible that changes in the protein synthesis in this muscle are not reflective of changes in this process in combined hind-limb muscles. Also, if the increase in urinary NMH excretion caused by adrenalectomy resulted from enhanced degradation of the gut NMH pool instead of the muscle NMH pool, the cimaterol-dependent reduction of urinary NMH in adrenalectomized animals may have resulted from reduced gut NMH release and therefore would not have resulted in a change in muscle weight.

Earlier we mentioned that cimaterol may enhance growth by antagonizing growth-inhibiting actions of glucocorticoids. This was supported by the ability of cimaterol to reduce the Dex-dependent increase in urinary NMH excretion. However, a similar antagonism was not seen in sham-control animals. Cimaterol did not antagonize the

glucocorticoid-dependent reduction in protein synthesis in sham-operated and Dex-treated animals and did not reduce urinary NMH excretion in sham-operated rats. Hence, the mechanism by which cimaterol enhanced muscle growth in sham-operated animals has not been elucidated by this study. It should be noted that there was a tendency for protein synthesis to be increased in epitrochlearis muscle; however, this muscle may not be representative of the effects of cimaterol on the hind-limb muscles we weighed. It is possible that the changes in protein synthesis caused by cimaterol were greater in hind-limb muscle. Rates of protein synthesis in biopsied muscle are considerably lower than those observed in vivo. Hence, the methods were used may not have been sensitive enough to detect statistically-significant changes in protein synthesis. There is evidence that anabolic effects of β-adrenergic agonist may be directed more specifically to synthesis of myofibrillar protein (Helferich et al.,1988). Our method assessed synthesis of all muscle protein and particularly the non-myofibrillar protein whose synthetic rate exceeds the rate of synthesis of the myofibrillar protein (Millward and Bates, 1983). Lastly, effects of cimaterol on protein synthesis may be directly and acutely mediated. Biopsy and incubation of muscle samples over 2 hours may have resulted in loss of effects of cimaterol on this process. It is possible that examination of the effects of these treatments using methods of assessing synthesis of specific muscle proteins in vivo could have revealed a mechanism by which cimaterol increased muscle weights in sham-adrenalectomized rats.

To study mechanisms by which changes in urinary NMH excretion may be mediated we examined activities of several proteolytic enzymes as previously mentioned. Cimaterol

only reduced NMH excretion in Adx rats on day 7 but neutral proteinase activity was reduced by cimaterol irrespective of glucocorticoid status. This implies that regulation of activity of neutral proteinase is not general mechanism by which cimaterol decreased urinary NMH excretion. The role of the neutral proteinase in muscle has been studied for many years. It was reported to be activated in human muscle diseases (Kar and Pearson, 1980) and since little work on this enzyme has been conducted in skeletal muscle. Recently, neutral proteinase activity has been attributed to an ATP-dependent proteolytic mechanism by Driscoll and Goldberg (1989) who believe this mechanism to be of particular significant to degradation of myofibrillar protein. Of interest was the ability of cimaterol, which has been reported to decrease myofibrillar protein degradation, to decrease neutral proteinase activity. This implies that regulation of myofibrillar protein degradation by cimaterol may involve control of these neutral proteinase activity. The response of cathepsin B activity to cimaterol was opposite to the response of neutral proteinase. Cathepsin L activity was increased by cimaterol in sham-operated and Dex-treated animals. Cathepsin D activity was increased in adrenalectomized rats and reduced in Dex-treated rats by cimaterol, however, urinary NMH was reduced on day 7 in adrenalectomized rats and not affected in Dex-treated rats.

Changes in muscle neutral proteinase and cathepsin B, D and L caused by cimaterol did not match the pattern of urinary NMH excretion caused by same treatments. This implies that other muscle proteinases could be responsible for the changes in urinary NMH excretion we detected. Alternatively, these enzymes may be important to the changes in NMH excretion we detected by which regulation of protein degradation by

these enzymes is directed towards compartmentation of substrates with these enzymes rather than towards regulation of their activities.

Activities of both mCDP and μCDP were reduced and calpastatin was increased by cimaterol in sham-controls. Forsberg et al. (1989) also reported that cimaterol reduced activities of mCDP and μCDP in muscle tissue taken from rabbit. Wang and Beermann (1988) and Higgins et al. (1988) also reported reduction of μCDP activity in sheep muscle caused by cimaterol and clenbuterol, respectively. However, these two studies reported that mCDP activity was increased. Contrary to their studies, we observed a reduction in mCDP activity. Cimaterol caused a reduction in urinary NMH excretion on sham-controls; however this effect was not significant. Others have reported a reduction in urinary NMH excretion (Forsberg et al., 1989) or a reduction in calculated muscle protein degradation (Reeds et al., 1986; Bohorov et al., 1987) in response to dietary β-agonists. Responses of μCDP, mCDP and calpastatin to cimaterol treatment suggests that regulation of calcium-dependent proteolysis may be a mechanism by which cimaterol regulate muscle protein accretion of sham-controls.

Higgins et al. (1988) in the same study also reported that activity of calpastatin was increased by clenbuterol which is supportive of our study. There is evidence that calpastatin plays a central role in regulation of CDPs activity in cells (Suzuki et al., 1988). It inhibits translocation of CDPs to membrane, removes both inactive and active forms of CDPs from membranes, and inhibits autolytic activation of CDPs (Suzuki et al., 1988). Hence, we have evidence that cimaterol-dependent muscle hypertrophy is associated with regulation of calcium-dependent proteolysis in sham-controls. This

regulation may be more specifically directed towards calpastatin. However, the observation that cimaterol increased activities of CDPs in Adx and AdxD animals raises some doubts about the role of calcium-dependent proteolysis in cimaterol-dependent muscle hypertrophy. Despite the fact that cimaterol effects on CDP activities were dependent upon glucocorticoid status, muscle calpastatin was increased by cimaterol irrespective of glucocorticoid status. Calpastatin activity was sufficient to completely inhibit CDP activity irrespective of cimaterol or glucocorticoid status. These data provide further evidence that calpastatin plays a central role in control of calcium-dependent proteolysis.

EFFECTS OF GLUCOCORTICOIDS AND CIMATEROL ON OTHER TISSUES

Cimaterol did not influence liver weight irrespective of glucocorticoid status; however, glucocorticoids appear to maintain liver weights, because adrenalectomy reduced liver weight in control-fed rats and Dex treatment restored it. Liver protein synthetic capacity (RNA concentration) was increased by adrenalectomy and restored by Dex treatment. These results agree with Odedra et al.(1983) who reported that corticosterone reduced liver RNA concentration in rats. The reduction of liver weights by adrenalectomy may result from enhanced protein degradation or from reduced liver protein synthetic efficiency. In support of the latter possiblity Odedra et al. (1983) reported that corticosterone increased slightly liver protein synthetic efficiency.

The reduction of liver weights by adrenalectomy was associated with enhanced

neutral proteinase activity. This change may, in part, be responsible for changes in liver weight caused by variable glucocorticoid status. Changes in activities of cathepsin B and D could not account for changes in liver weights caused by variable glucocorticoid status.

Liver had higher activities of cathepsins B, D and L compared with muscle, however, its neutral proteinase activity was very low compared to muscle. This implies that the cathepsins may be more important to degradation of liver proteins compared to muscle and that neutral proteinase is more important to degradation of muscle protein than degradation of liver protein. Many other authors have reported that activities of the cathepsin are greater in liver than muscle (Forsberg and Wehr, in press). Mortimore (1982) has reported that the lysosomal cathepsins are responsible for the bulk degradation of liver proteins.

CONCLUSION

Adrenalectomy reduced weight gain which resulted from reduced feed intake, and dexamethasone treatment further reduced weight gain which resulted from decreased feed efficiency. Cimaterol stimulated weight gain of Sham and AdxD animals but not of Adx animals. The response of hind-limb muscle weight to variable glucocorticoid status and to cimaterol was similar to the response of weight gain to the same treatment. Since adrenalectomy and dexamethasone treatments reduced both weight gain and muscle weights, this suggests that certain levels of glucocorticoids are required for optimal growth.

The glucocorticoid-dependency for acute anabolic actions of cimaterol was not confirmed in this study because cimaterol-fed animals receiving Dex treatment did not gain faster than cimaterol-fed adrenalectomized animals. Instead, we proposed that ability of cimaterol to enhance growth and muscle werght in Sham and AdxD animals relates to its ability to antagonize the growth-inhibiting actions of glucocorticoids. This antagonism was evident in protein degradation of AdxD rats as indicated by urinary NMH excretion which was reduced by cimaterol treatment; however, this antagonism was not evident in muscle protein synthesis as indicated by protein synthesis of isolated epitrochlearis muscle.

Adrenalectomy had a tendency to increase protein synthesis in isolated epitrochlearis muscle on day 8 of the study, however, only in cimaterol-fed animals was this effect significant. Cimaterol slightly increased protein synthesis irrespective of glucocorticoid

status on day 8 of the study, although, none of these effects were statistically significant. Therefore, we have no evidence that changes in weight gain and muscle weight caused by variable glucocorticoid status and cimaterol were due to the changes in protein synthesis. Rather muscle protein degradation may be responsible for these changes. This was supported by the observation of urinary NMH excretion which was enhanced several-fold in Adx and AdxD animals and was decreased in cimaterol-fed animals. However, protein synthetic efficiency (protein synthesis/RNA) and protein synthetic capacity (RNA concentration) of isolated epitrochlearis muscle were affected by glucocorticoid status and cimaterol in opposite direction (i.e. glucocorticoid and cimaterol increased muscle protein synthetic capacity yet decreased muscle protein synthetic efficiency).

Urinary NMH was increased by adrenalectomy and was reduced by Dex-treatment on day 7 of the study. Cathepsin L activity was increased slightly by Adx and was reduced by Dex treatment thus resembling the pattern of urinary NMH excretion caused by variable glucocorticoid status. However, the magnitude of changes in cathepsin L activity did not approach the magnitude of changes in urinary NMH excretion on day 7. Neutral proteinase activity was increased by Adx but was not affected by Dex treatment. Cathepsin D activity was opposite the response of cathepsin L. Activities of both mCDP and μCDP were reduced by Adx, and Dex treatment did not further reduce activities of mCDP and μCDP. Calpastatin activity was not affected by glucocorticoid status. Cimaterol only reduced NMH excretion in Adx rats on day 7 of the study, however, neutral proteinase was reduced by cimaterol irrespective of glucocorticoid status. This implies that regulation of NSP is not general mechanism by which cimaterol reduced

NMH excretion. Cathepsin B response to cimaterol opposite the direction of neutral proteinase. Cathepsin L was increased by cimaterol in Sham and AdxD rats. Cathepsin D activity was increased in Adx rats and reduced in AdxD rats by cimaterol. Both mCDP and μCDP activities were reduced in Sham and increased in Adx and AdxD animals by cimaterol treatment. Calpastatin activity was increased by cimaterol irrespesctive of glucocorticoid status. If changes in urinary NMH excretion identified in this study have arised solely from changes in degradation of muscle protein, it is unlikely that alterations in muscle cathepsins, neutral proteinase and CDPs could account for the magnitude of changes in NMH excretion. This implies that other muscle proteinases could be responsible for the changes in urinary NMH excretion we detected. Alternatively, these enzymes may be important to the changes in NMH excretion we detected by which regulation of protein degradation by these enzymes is directed towards compartmentation of substrates with these enzymes rather than towards regulation of their activities.

We noticed, however, calcium-dependent proteolysis especially regulation of calpastatin may be a mechanism by which urinary NMH excretion was mediated by cimaterol in Sham animals. Because urinary NMH excretion of Sham was reduced 40 % by cimaterol whereas calpastatin (the endogenous inhibitor of CDPs) activity was increased by 37 %. Moreover, mCDP and μCDP activities of Sham were reduced by cimaterol 15 % and 30 %, respectively.

Although, this study did not completely elucidate the mechanisms by which cimaterol exerted anabolic actions in variable glucocorticoid status, it provides us a good model to examine muscle protein degradation. In the future, studies should be conducted

to reveal the involvement of other proteinases and the cellular compartmentation of muscle proteinase in control of muscle protein degradation.

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