Chapter 1

Introduction

One of the intercellular communication is termed as "endocrine" in which cells influence by other cells releasing into circulating body fluid particular chemicals called hormones The word "hormone" derives from Greek word means "to arose to activity" Although the hormones come into contact with every cell in the body, they only affect those target cells, which possess the specific receptors for a particular hormone (1).

The hormones are broadly divided into three groups

1 Peptide hormone

2 Steroid hormone and .

3 Biogenic amines

The other control systems are nervous system The endocrine and nervous systems both appear early in the developmental period as well as evolutionary scale

The role of thyroid, sex, growth and adrenal glucocorticoids in neural ontogeny has been reviewed by Jacobson (2)

The "Introduction" Chapter 1 focuses on the literature concerning glucocorticoids Only corticosterone, cortisol, cortisone and 11-dehydrocorticosterone have appreciable glucocorticoid activity Several synthetic compound are known to possess glucocorticoid activity e g dexamethaosone, methylprednisone, and triamcinolone (3)

Historical Background

The clinical symptoms associated with reduction of steroid hormones have been known for a long time In 1855, Addisson accurately described the symptoms of adrenal insufficiency In 1889, Brown-sequard, eventually isolated the active steroids In 1920, the vital importance of

adrenal cortex was appreciated and the distinctions were made between the hormones secreted by the adrenal cortex and medulla The first success was reported in 1929, when Doisy and Butenandt crystallized estrone from the urine of pregnant women Corner later discovered progesterone Richstein, in the 1930s identified corticosteroids (1) In 1948, cortisone was made from bile acids in sufficiently larger quantity for clinical trials, and its power to induce remission of rheumatoid arthritis was demonstrated, but in 1950s it was realized that cortisone is biologically inert and the active natural hormone is hydrocortisone (cortisol) Pincus demonstrated the contraceptive possibilities of estrogen and progestin preparation These results established steroid hormones as potential drugs, beyond simple use in replacement therapy This led to in extensive chemical and pharmacological studies Shortly thereafter, the introduction of "radioactive steroids" of high specific activity enabled the first efficient studies of the mechanisms involved in hormone action Shortly, thereafter, the discovery of steroid hormone receptors followed The use of the same labeled steroids in "competitive binding assays" permitted the direct measurement of plasma concentrations of hormones Clinical investigation of endocrine disorders was changed completely from that point onwards (1)

Adrenal gland

Adrenal gland has two distinct regions i e adrenal cortex, which is derived from mesoderm present as outer region and 2 Adrenal medulla, which is derived from ectoderm present as inner region At birth fetal adrenal cortex represents the major proportion of the adrenal mass and it regresses rapidly and disappeared by the end of the first year of life The definitive or adult adrenal cortex develops, but its characteristic differentiation into three Zone is completed only by the third year of life (1)

low affinity and high capacity CBG can bind with cortisol, corticosteroid, 11-desoxycorticosteroid and 17 hydroxy progesterone with high affinity. Among the synthetic steroids, prednisolone is bound whereas dexamethasone and triamcinolone are not bound with CBG Plasma concentration of CBG is approximately 30 mg/lit (1) The concentration of CBG increases during pregnancy and during treatment with estrogens (contraceptives, treatment of cancer, prostate etc) The SBP binds C_{19} or C_{18} steroids of planar structure and C_{17}-β hydroxy steroids The metabolites of testosterone, testosterone itself and estradiol bind with decreasing order of affinity Its normal plasma concentration is 3 mg/lit About 10% of the hormone is in the free form, which is biologically active Half-life of corticosterone is 50-90 min as reported by Hardy, 1984 (4) The concentration of albumin is approximately 1000 to 10000 times greater (40 g / lit) than that of specific binding protein It binds all steroids with a dissociation constant (Kd) between 1 μM (estrogen) and 1 mM (cortisol) The affinities are inversely proportional to the polarity of the steroids In humans, daily production of adrenal steroid is 25-35 mg of which 80-90% (20-30mg) is cortisol and 5 to 10 % (2-4 mg) is corticosterone In rats, the major glucocorticoid is corticosterone with a plasma concentration is 4-25 μg/dl In humans, cortisol level in plasma is 7-25 μg/dl The inactivation of glucocorticoid occurs mainly in liver and metabolized derivative are conjugated with glucuronic acid and can be readily excreted in urine (8)

Factors affecting glucocorticoid levels

Diurnal fluctuation

Glucocorticoids i e cortisol and corticosterone follows day and night variation which is of neural origin and involves parallel fluctuation in ACTH and CRF release The lowest plasma corticosterone level in rats is seen early in the morning hours while peak level is reported at the onset of darkness (9,10)

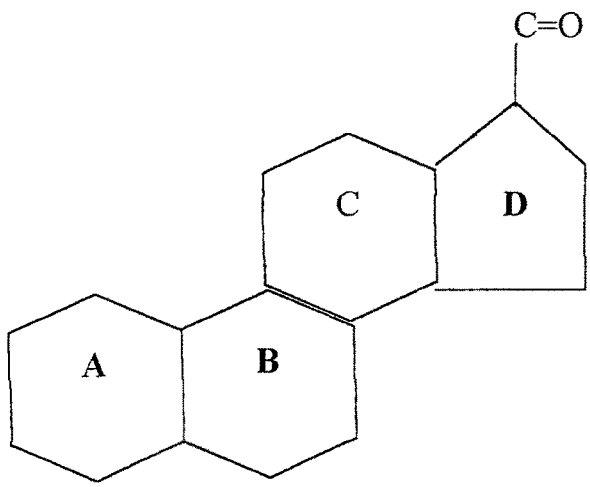
The definitive adrenal cortex consist of three zones The cells of Zona glomerulosa are arranged in circles and secrete aldosterone In of Zona fasciculate, which is the largest zone, the cells are arranged longitudinally, in columns and synthesize and secret glucocorticoids (GC) and the cells closest to medulla form zona reticularis that secrete androgens The zona fasciculata and zona reticularis are under the control of ACTH (1,4)

All steroids are derived from 17- carbon cyclopentanoperhydrophenanthrene structure with four ring labeled as A to D (Fig, 1) (1)

Biosynthesis of steroid hormones

In steroid forming glands, biosynthesis begins with cholesterol After internalization, cholesterol is released and immediately utilized or stored in the form of esters in the ctyoplasmid lipid droplets Side chain cleavage of cholesterol occurs in mitochondria It involves a complex enzymatic system of hydroxylation of C_{20} and C_{22}, followed by the action of desmolase The resulting compound is pregnenolone ACTH regulates this reaction in adrenals Pregnenolone is oxidized at C3, resulting in ketone function, and isomerization of double bond occurs forming the conjugated ketone or progesterone A series of hydroxylation follows at position at C11- (11-deoxycortisol) and then C11 β forming corticosterone The corticosterone is further hydroxylated at C18 followed by oxidation of alcohol group to aldehyde, resulting finally to functional aldosterone which act as a major minerealcorticoid in humans The pregnenolone ring may be converted into 17-hydroxypregnenolone with the help of enzyme 17-alpha hydroxylase followed by series of hydroxylation at C21 and C11 β which previously mentioned, the product formed is cortisol which is the major glucocorticoids In humans hydroxylation at C17 does not occur for corticosterone, the production of which is 10% of the cortisol in humans In rat and mice, this

1. 17 Carbon cyclopentanoperhydrophenenthrene ring structure for Steroid hormones



enzyme 17α hydroxylase is practically absent and therefore corticosterone is the only glucocorticoid in these species Thus the zona fasciculata and zona reticularis possess all enzymatic equipment necessary for the synthesis of cortisol and adrenal androgens The zona glomerulosa, deficient in 17-hydroxylase and possessing 18-hydroxylase, synthesizes mineralocorticoids (4)

Regulation of glucocorticoid synthesis

Glucocorticoids are released into circulation as soon as they are synthesized and only trace amounts can be detected in the adrenal gland Thus, the rate of secretion is determined by the rate of synthesis

The synthesis of glucocorticoid is controlled by HPA axis (Hypothalamus-Pituitary-Adrenal axis) The biosynthesis of glucocorticoid is controlled by ACTH and secretion of ACTH is regulated by CRF (Corticotropin Releasing Factor) CRF is itself under dual control, long loop feedback control regulated by the level of free cortisol and regulation by higher brain centers and a short loop feedback by the concentration of ACTH has also been proposed Also, cholinergic secretion and CNS stimulus negatively regulate CRF secretion Epinephrine, norepinephrine and GABA positively regulate CRF secretion (5-7)

Circulation, Transport and Metabolism

Steroids are transported primarily in the plasma where they are bound to proteins called steroid binding plasma proteins which are specific as well as nonspecific plasma proteins CBG (Corticosterone Binding Globulin) and SBP (Sex Hormone Binding Protein) are known as specific binding proteins because they bind only with certain steroid with high affinity and low capacity Albumin is said to be non-specific because this protein binds all steroids with

Fig. 2 Regulation of Corticosterone Synthesis

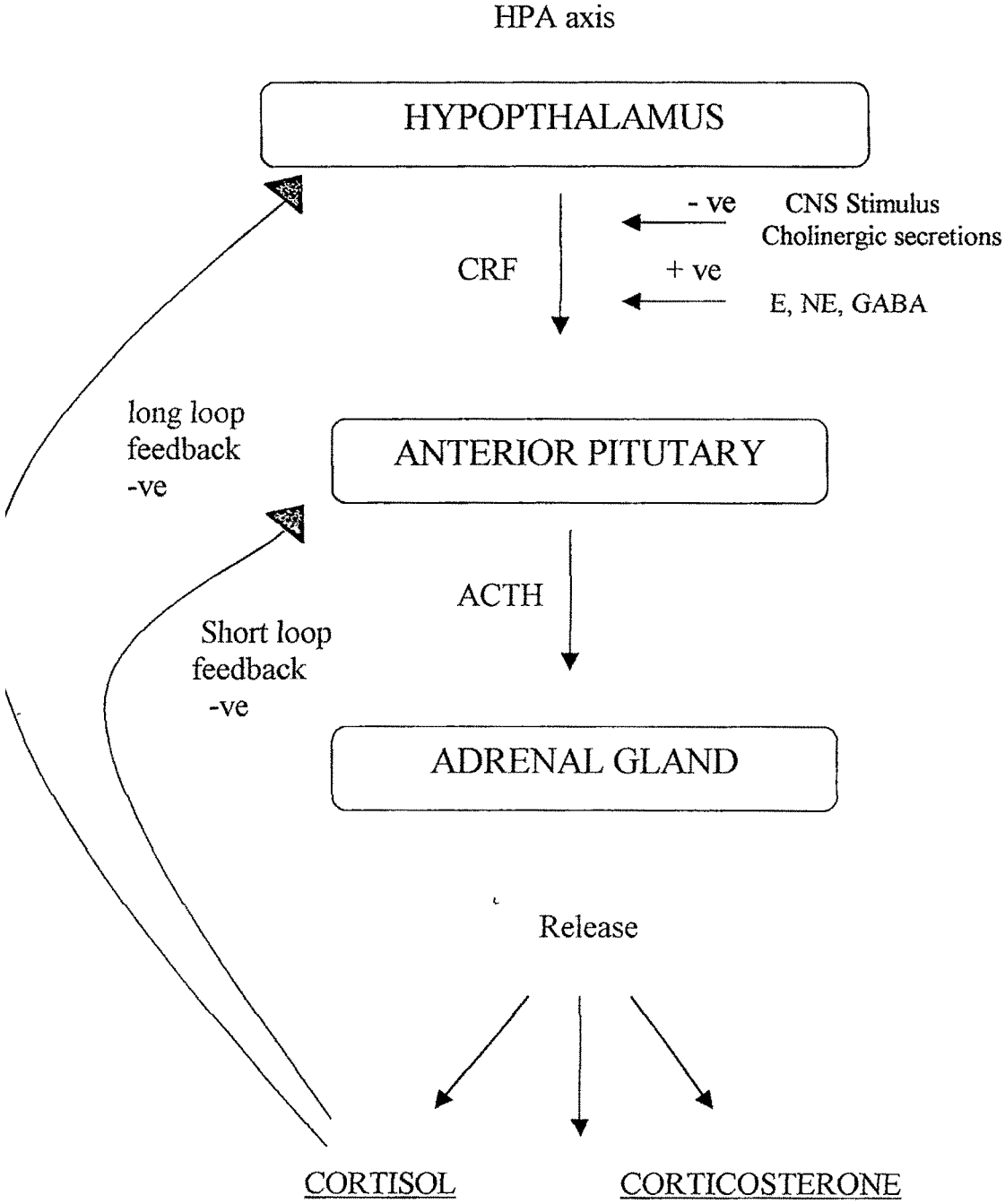
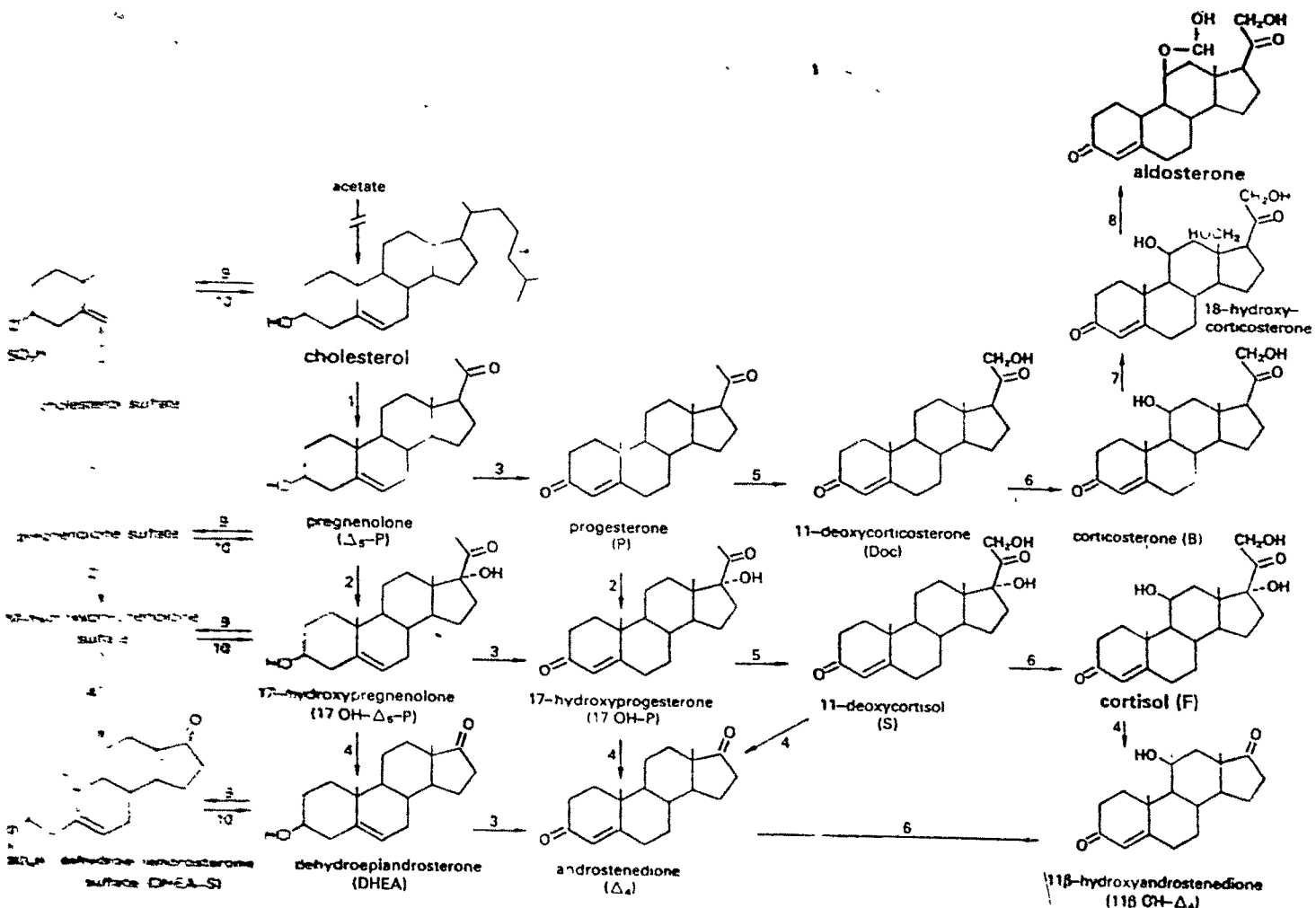


Fig. 3 Biosynthesis of steroid hormes.



Glucocorticoid level in plasma show seasonal variation during the course of the year as reported by Ahlersova et al (11) Glucocorticoid levels in plasma show age-dependent variation (12,13) The plasma level of corticosterone in rats is high before birth and falls immediately after birth and remains low till day 14 of postnatal life and after that it begins to increase and reaches the adult value as reported by Sapolsky and Meaney (13) This period is known as Stress Non Responsive Period (SNRP) Wide range of stress conditions such as heat, surgery and cold stress are known to elevate plasma cotrticosteroid levels from 1-3 fold to 10 fold as compared to age-matched controls and the extent of increase was comparatively less in animals of age less than 15 days (13) Circulating levels of glucocorticoids could also be affected by some other factors as reported by Przegalinski et al (14)

Molecular mechanism of glucocorticoid action

Glucocorticoids exert both genomic as well as nongenomic action

Genomic action of glucocorticoids

Being lipophilic in nature, glucocorticoids could easily gain entry into target cells by diffusion across the plasma membrane After entering into target cells, glucocorticoid hormones bind to their specific receptors present in cytosol Upon binding to hormone, glucocorticoid receptor undergoes a conformational change, which enables the hormone-receptor complex to get translocated into nucleus This hormone –receptor (HR) complex binds to specific acceptor sites of the DNA and thereby modulated the expression of target genes in a tissue specific manner resulting in a cascade of biological events (15-17)

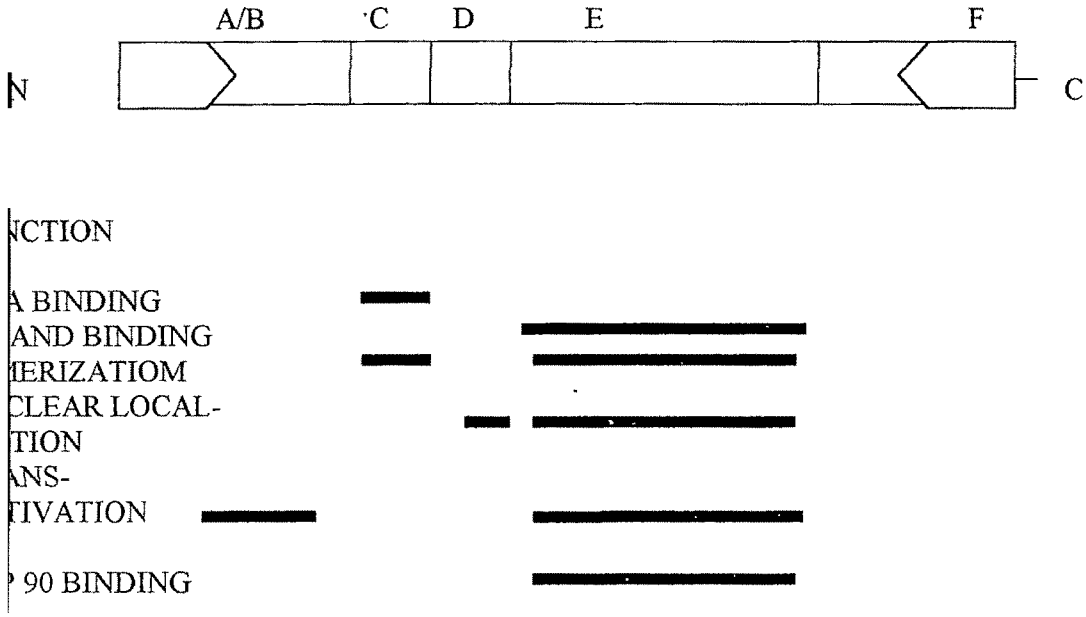
Glucocorticoids can alter overall rate of RNA synthesis and also processing of RNA They can activate synthesis of specific messenger RNA (mRNA) by the transcriptional and post transcriptional processes which regulate several enzymes of metabolic pathways in different tissues including brain (15-18)

The modulation of gene expression by glucocorticoids could be either by induction or repression mechanism Many of the enzymes of the metabolic pathways are inducible by glucocorticoids (3,15-18) Glucocorticoids induce a protein named macrocortin (lipocortin) which inhibits phospholipid hydrolyzing enzyme phospholipase A_2, this is an example of inhibition of enzyme (19,20) Adrenolectomy leads to significant reduction of mRNA levels of transcortin (21) Similarly a plasminogen activator (serine protease) has also been reported to be inhibited by an inhibitory protein induced by glucocorticods (22)

The glucocorticoid receptors belong to the superfamily of steroid nuclear receptors (23) Fig shows structural and functional organization of steroid nuclear receptors The glucocorticoid receptor protein has been characterized and purified from different sources It is a single polypeptide with molecular weight 90 to 100 Kda (24) Proteolytic treatment shows that the receptor is composed of different functional domains (25)

The DNA binding domain is the best conserved among the members of the receptor superfamily It consists of about 70 amino acids that fold into two zinc finger motifs Each finger binds to a zinc ion that is tetrahedrally coordinated by conserved cysteines The first finger (NH_2 terminal) which determines response element specificity contains several hydrophobic amino acids apart from four cysteines The second finger (COOH- terminal) consists of five cysteine and many basic amino acid residues and is involved in protein-

Fig. 4 Structural and functional organization of steroid nuclear receptors.



protein interactions such as receptor dimerization (26-27). The second best conserved domain of receptors is hormone binding domain, which binds to the ligand in hydrophobic pocket and participates in other functions such as nuclear translocation, receptor dimerization and transcriptional activation (26,28-30)

Glucocorticoid receptors also posses binding site for heat shock protein (hsp 90) that appears to modulate the subsequent response to hormonal signal (30) Heufelder et al (31) have studied receptor mediated glucocorticoid effects on expression and synthesis of heat shock protein hsp-72 ie thought to play a role in thyroid autoimmunity An additional independent transcriptional activation function is locate within NH_2-terminal of the receptor, which possesses a marked cell type and promoter specificity (32)

The function of glucocorticoid receptor in normal cells is regulated by a hormone and ATP dependent phospphorylation/-dephosphorylation cycle, which governs hormone binding (33, 34) and translocation of hormone-receptor complex to nuclei (35) Cyclic AMP decreased while cyclic GMP facilitated glucocorticoid binding to its receptors, and this binding is dependent on ATP and divalent cations (36) Phosphorylation of glucocorticoid receptors occurs at NH_2-terminal domain (37) by protein kinase A or proline directed serine kinase (36, 38) Dephosphorylated form of glucocorticoid receptor lacked the ability to bind to DNA and results into inactivation of the receptor (39) Protein kinase C activators and inhibitors are also known to affect functioning of glucocorticoid receptors (40-42) Denielsen et al (43) have shown that phosphorylation of glucocorticoid receptor increase the binding of many transcriptional regulator proteins to DNA Besides phosphorylation, other post- translational modifications such as glycosylations and acylation of gucocorticoid receptor can modulate receptor function indirectly by affecting the phosphorylation of receptors (44-46)

It has been shown by various researchers that the fatty acids can modulate glucocorticoid receptor function (47, 48) Polyunsaturated fatty acids have been shown to decrease the binding of [^{3}H] dexamethasone to glucocorticoid receptors in rat liver the inhibition of binding is of mixed non competitive type, suggesting that these fatty acids bind at a site on receptor different from the hormone binding site (49)

Sato et al (50) have reported down regulation of glucocorticoid receptor gene expression and decreased number of glucocorticoid receptors by lipoprotein LP(a) in human smooth muscle cells However , low, very low and high density lipoprotein have no effect on glucocorticoid receptors

Radio ligand binding studies have demonstrated that corticosteroids act through two types of receptors in brain These receptors are referred to as type-1 which is mineralocorticoid type (MR) in its binding properties and the type-2, which is identical with glucocorticoid receptor (GR) (51-56) This resolution into two central corticosteroid receptor systems is characterized by a difference in steroid specificity and neuroanatomical distribution MR binds the endogenous glucocorticoids of rat-corticosterone – as well as mineraocorticoid aldosterone with high affinity, and these receptors are widely distributed in kidney and other peripheral tissues In brain, MR is predominantly localized in septum and hippocampus (53,55,57-59) in contrast , GR show high affinity for the potent synthetic analogs dexamethasone and RU 28362 (55, 60) These GR are widely distributed in the rat brain (55,61-66) and throughout other tissues also Spleen and thymus also contain GR in very high concentrations (67) In rat hippocampus both types of the receptors –MR and GR –are expressed (68)

Crticosterone binds to both MR and GR, but binding affinity to GR is 6-10 times lower than MR At a very low dose of corticosterone (1 µg/100g body wt) 80% of GR gets occupied but for 95 % occupation of GR a dose of 1 mg/100g body wt is or corticosterone in required (55) Brain MRs bind cortisol with high affinity, but low capacity and are therefore largely occupied under basal conditions, whereas GRs are unoccupied under basal conditions because of low affinity, but become occupied during stress (69, 70) The differential occupation of these two receptors by corticosterone may be important for role of corticosterone in control of brain function during different physiological or pathological conditions (55, 71)

In brain, levels of both MR and GR are regulated by glucocorticoid status Adrenalectomy upregulates while repeated stress downregulates GR in frontal cortex and amygdala (73-74) Spencer et al (75) have reported that corticosterone down regulates both the receptors MR and GR in brain regions , pituitary and immune tissues In case of hippocampus, glucocorticoids autoregulates GR mRNA levels and hormone binding sites in short term, but probably not in the longer term, whereas MR are little effected by glucocorticoids (69, 76-78)

The paradox essentially boils down to the different in vivo vs in vitro binding properties of dexamethasone in mineralocorticoid receptors De kloet et al (79) have reported that [3H] dexamethasone in contrast to [3H] corticosterone was not taken up and retained by mineralocorticoid in the hippocampal neurons of adrenolectomized rat However in vitro dexamethasone has been shown to bind with high affinity Kd 0 5 to 1 nm to hippocampul MR in different species In vivo dexamethasone exerts hardly any agonistic effects via

kidney or brain MR receptor and might even act as MR agonist in brain One factor that limit the access of dexamethasone to MR is the mdr1a-P-glycoprotein which is expressed in the apical membranes of endothelial cells of the blood brain barrier (BBB) (80) This protein acts as an energy dependent pump removing xenobiotic substances including synthetic steroids from the brain parenchyma (81) In addition this protein (mdr1s P-glycoprotein) although present in the kidney does not show the removal of dexamethasone from the organ (81)

Nongenomic Effects

Variety of steroid hormones including glucocorticoid are known to have several nongenomic effects These have reviewed by Duval et al (82) Rapid effects of glucocorticoids on excitable membrane may be due to alteration in the characteristics of the membrane by intercalating onto phospholipid bilayer Accumulation of glucocorticoids within neuronal membrane can alter binding characteristic of neurotransmitter to their receptor and also the gating characteristic of ionic channels (83,84) In synaptic plasma membrane glucocorticoids evokes an increase in Na^+, K^+ ATPase activity (85,86) have shown high affinity and low capacity glucocorticoid binding sites in kidney plasma membranes, similar glucocorticoid binding sites are also present in liver (87-89) and synaptic plasma membrane (90-91)

Glucocorticoid also alter calcium homeostasis in hippocampal neurones leading to increase calcium concentration and hyperactivation interrupting memory related process (92-93)

Physiological and behavioral effect of Glucocorticoids

Glucocorticoids have numerous physiological and behavioral effects and play an important role in maintenance of various physiological processes and regulation of behavior

Anti-inflammatory effect

Glucocorticoids are widely used as anti inflammatory agents because of their immunosuppressive action They inhibit allergic and inflammatory action in several ways Glucocorticoids are known to stabilize lysosomal hydrolytic enzymes (82) Glucocorticoid decreases permeability of blood capillaries to leucocyte and thus circulating lymphocytes, eosinophils, monocytes and basophils are reduced Glucocorticoids also decrease humoral type immune responses by reducing antibody production Many of the anti-inflammatory action of the glucocorticoids appear to be regulated by their capacity to inhibit the release of arachidonic acid from lipids (95) This inhibition has been demonstrated to be mediated through induction of phospholipase inhibitory protein (lypocortins) by glucocorticoids (96) Duval (97) has reported that dexamethasone is able to inhibit the transformation of arachidonic acid into prostaglandin and also blocks its acylation of phospholipid in the isolated mouse thymocyte (96)

Besides anti-inflammatory effects of glucocorticoid show anti-growth effects, effects of parturition as well as also effects on mood, motivation and behavior patterns

Metabolic effects of glucocorticoids

Carbohydrate metabolism

Primary role of glucocorticoid is to maintain blood glucose level and reserves of glycogen in the liver and to a lesser extent in heart and skeletal muscles Glucocorticoids promote

gluconeogenesis from amino acids and glycerol Glucocorticoids induce enzymes involved in gluconeogenesis such as glucose 6 phosphatase, fructose, 1,6 biphosphatase and phosphoenolpyruvate kinase (PEPCK) in the liver (4,8,98) Glucocorticoids also block glucose transport in certain brain region like hippocampus under both *in vitro* and *in vivo* conditions (99-100). Thus, glucocorticoids exert an anti-insulin effects by blocking uptake of glucose by extra hepatic peripheral tissues Glucocorticoids seem to decrease glucose transporters by causing translocation in plasma membrane thereby decrease glucose uptake

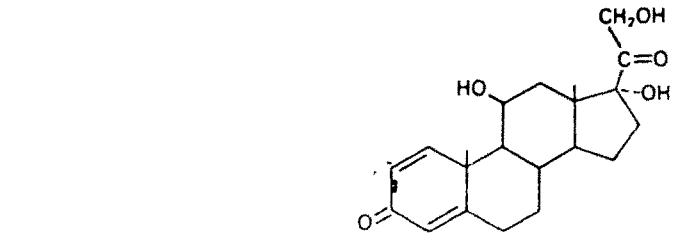
Protein and amino acid metabolism

Glucocorticoids enhances release of amino acids from protein in the skeletal muscle and other extrahepatic tissues including the protein matrix of bone The increased gluconeogenesis by glucocorticoid from amino acids is associated with the urea production via conversion of amino nitrogen to urea Adrenolectomized rat showed decrease in urinary nitrogen excretion which was restored upon glucocorticoid treatment (101) Besides increasing protein breakdown in extra hepatic tissues, glucocorticoids also reduce amino acid uptake and protein synthesis in brain, muscle and other tissues The skeletal muscle breakdown was increased by glucocorticoids (102) In liver, glucocorticoids showed anabolic effects and also induced enzymes involved in amino acid metabolism e,g tyrosine transaminase, tryptophan pyrolase

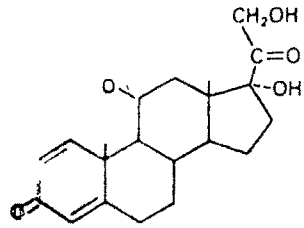
Lipid metabolism

Glucocorticoids are primarily lipolytic hormones and their lipolytic effect is in part due to potentiation of lipolytic action of other hormones such as growth hormone, glucagon, catecholamines and thyroid hormones It releases free fatty acids from the adipose tissue which are mobilized to liver Melby *et al.* (103) have reported glucocorticoid induced

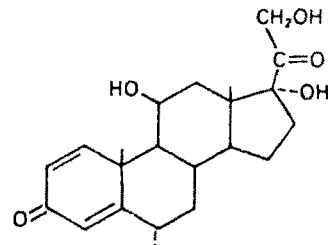
Fig. 5. Structures of synthetic steroids.



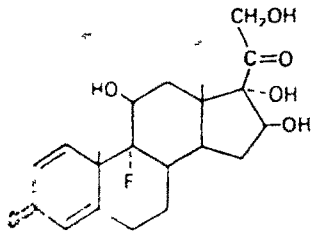
prednisolone



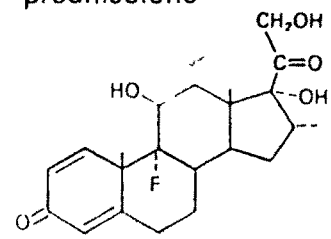
prednisone



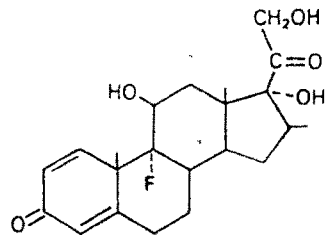
methyl- CH_3 prednisolone



triamcinolone



dexamethasone



betamethasone

changes in lipid and phospholipid in rat liver microsomes in fibroblasts cell line. Dexamethasone causes an increase in sphingomyelin content by inducing the enzyme biosythetic pathway (104) Kaur *et al.* (105) have reported effects of dexamethasone treatment on metabolism of neutral lipids and phospholipid in various rat tissues including live, kidney, testis and heart

Pharmacology of Glucocorticoids

Compounds having different effects on inflammation, salt retention are obtained by various chemical substitution in the original structures of cortisol and corticosterone The induction of double bond between C_1 and C_2 increases anti-inflammatory activity In contrast, substitution at C9α by fluorine increases both activity The effect of double bond at position 1 and fluorine at C9α is additive The 16α OH, 16α or β methyl substitution showed marked reduction in salt retention Thus, various synthetic steroids come into the market with variable anti-inflammatory activity, glucocorticoid activity or mineralocorticoid activity The relative potencies are as given in Table 1

As shown in Table 1, dexamethasone is one of the most potent, predominantly used synthetic steroids It is used in all age groups due to its availability in four different formulations like oral, elixir, ointments and injections (94)

Therapeutic uses of dexamethasone

Glucocorticoids used as a replacement therapy in acute and chronic adrenal insufficiency The therapy is also useful in congenital adrenal hyperplasia in which one of the several enzymes required for biosynthesis of steroid hormone is deficient The synthetic glucocorticoid treatment showed usefulness in the treatment of a variety of rheumatic

le 1· Relative potencies of steroid hormones as compared with cortisone

oid	Glucocorticoid Effect (Anti- inflammatory)	Mineralcorticoid Effect (Sodium retention)	Equivalent Dosage (mg)
tisone	0.8	1.0	25
lrocortisone	1 0	1 0	20
amcinolone	5.0	None	4.0
xamethasone	30	Minimal	0.75
amethasone	30	Negligible	0.75
rocortisone	15	150	Irrelevant
osterone	None	500	-
ticosterone	0.2	2.0	-

disorders such as systemic lupus erythematosus and variety of vasculitis disorders such as polyarthritis nodosa, Weqner's granulomatosis and giant cell arthritis The glucocorticoid are useful in the several allergic diseases as well as in chronic obstructive pulmonary disease (COPD) Many physicians recommended inhaled glucocorticoids over treated with oral dose of theophylline in the treatment of children with moderately severe asthma, in part because of the behavioral toxicity associated with chronic thophylline administration Glucocorticoids clearly decrease the incidence of long term neurological impairment associated with Haemophilus influenzae type b meningitis in infants and children two months of age or older Glucocorticoids are frequently used for ocular diseases The topical administration of glucocorticoids to patients with bacterial, viral or fungal conjunctivitis can be useful and mask the progression of the injection The use of glucocorticoids for glaucoma is also recommended Glucocorticoids treatment are useful in variety of inflammatory dermatitis As a result, a large number of different preparations and concentrations of topical glucocorticoids of varying potencies are available Use of glucocorticoid treatment for gastro intestinal diseases like inflammatory bowel diseases (chronic ulcerative colitis and Crohn's disease) is recommended Glucocorticoids are used in chemotherapy of acute lymphocytic leukemia and lymphomas because of their antilymphocytic effeects Corticosteroids are of in the value in the reduction or prevention of cerebral edema associated with parasites and with neoplaslms especially those that are metastatic Glucocorticoids are also used for miscellaneous diseases like sarcoidosis, thrombocytopenia, autoimmune destruction of erythrocytes, organ transplatation as well as spinal cord injury In addition, to their therapeutic uses, glucocorticoids are used for diagnosis purposes In the Cushing's syndrome as well as ectopic ACTH production or in person with adrenocortical tumors, the dexamethasone suppression test is employed (94,106,107,108) The use of repeated courses of corticosteroids is prescribed in 98% of the cases by obstetricians (109,110) Whitelaw and

Thorenson (110) have stated that randomized clinical trials show that two injections of corticosteroids into the mother before preterm delivery reduce respiratory distress syndrome, neonatal mortality and intraventricular hemorrhage Single course corticosteroid treatment before preterm delivery must still be recommended as a life saving and cost-effctive intervention Liggins (111) carried out a large randomized controlled trial, which showed a reduction in respiratory distress syndrome and neonatal death after two intra-muscular injection of 12 mg betamethasone 24 hrs apart to mothers before preterm delivery Treating 23 mothers with corticosteroid before preterm delivery would prevent one neonatal death on an average (110)

From one course to multiple course of corticosteroids

Initial trials suggested that the beneficial effect of corticosteroids was absent if there was an interval of over 7 days between treatment and delivery This finding persuaded an increasing number of obstetriations to repeat the course of steroids after 7 days if the pregnant woman at risk of preterm delivery had not yet given birth A recent survey of British obstetric departments showed that 98% are prescribing repeated courses of corticosteroids (109) It is now not uncommon the pregnant women with twins receive six courses of corticosteroid as prophylaxis covering the period from 24-34 week gestation Both betamethasone and dexamethasone are recommended for use before preterm delivery by the American National Institutes of Health (110)

Toxicity of Glucocorticoids

The synthetic glucocorticoids are believed to have lesser or no side effects or toxicity However when used in pharmacological doses or for prolonged period, they can give rise to

adverse side-effects which are seen in as high as 50 % of the cases (94) In general the toxic effects are adrenal suppression, iatrogenic Cushing's syndrome, osteoporosis, diabetes, ketoacidosis, muscle wasting, thinning of skin, weight gain and fat deposition at the back of neck (buffalo hump) and at the face (Fullmoon face) The other complications are peptic ulcers, bacterial and micotic infections, myopathy, nausea, dizziness and weight loss in some cases, and psychosis may occur (94)

Effect of dexamethasone on growth and development

Glucocorticoids and Brain development

Glucocorticoids appear to exert multitude of effects of nervous systems The effects range from control of most basic processes of cellular growth and differentiation to alterations in electrophysiological activity and finally to subtle but yet important influences on mood motivation and learned behavioral patterns Field (112) appears to have published first reports that neonatal glucocorticoids administration to rats inhibits rat brain development (112) The most general effect is a long lasting decrease in cerebrum and cerebellar tissue weights in rats (113,114,115) These decreases in brain weight are accompanied by significant reduction in DNA content suggesting that fewer cells are present in the brains of glucocorticoid treated animals Biochemical studies showing decreased ornithine decarboxylase (116) and thymidine incorporation in to DNA (117,118) indicate a general suppression of cell proliferation with little effects of glucocorticiod treatment on cell loss (119) Glucocorticoid also affects the differentiation and development of neurotransmitter system Also glial cells differentiation is regulated by glucocorticoids The promotion of glial cells differentiation as it relates to glial enzymes GS (Glutamine Synthatase) in astrocytes and G3PDH (Glycerol 3 phosphate dehydrogenase) in oligodendroglial cells

Animal studies have also shown that maternal corticosteroids treatment delays myelination and reduces growth of fetal brain areas particularly the hippocampus

Thus, the glucocorticoid, especially synthetic steroids have pronounced effect on brain as well as liver

Aim and Scope

From the literature survey, it is clear that glucocorticoid administration has deleterious effects on various aspects of brain development The dexamethasone is the most potent anti--inflammatory and immunosuppressive glucocorticoid agonist used for several therapeutic purposes in practically all age groups The side effects incidences are as high as 50% of the cases (94,110) The development of human brain continues up to 2 years and exposure of steroid during this period can have adverse effect on the ongoing process of development

For studying the above aspects, biochemical investigation was carried out to find dexamethasone induced changes in rat brain metabolism This can be achieved by cellular macromolecules quantitation as well as its metabolic activity Also, the mitochondrial structure and function studies by studying oxidative phoshorylation, and lipid environment and enzyme activity can serve as the guideline for energy status of the brain Similar type of studies was also carried out for liver tissue as well as on mitochondria, which can serve as an internal control

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