UNIT-I

Methods for Assessing the Effects of Chemicals on the Endocrine System

Chemical Bioregulation in Physiological functions

Course No. – VPY- 609

Credit Hrs. – 3+0=3

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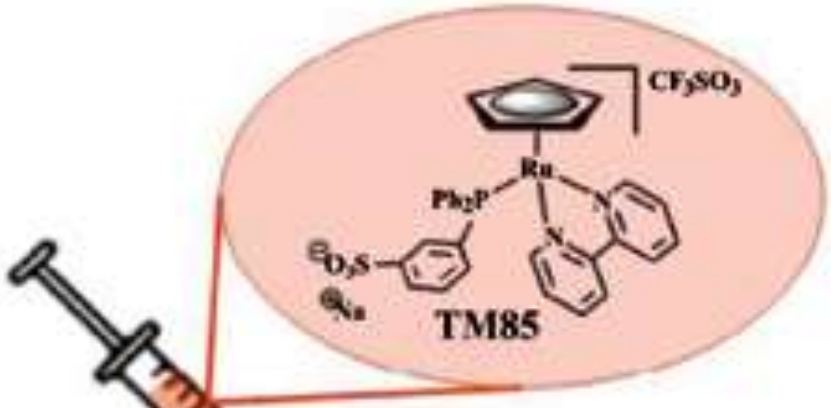
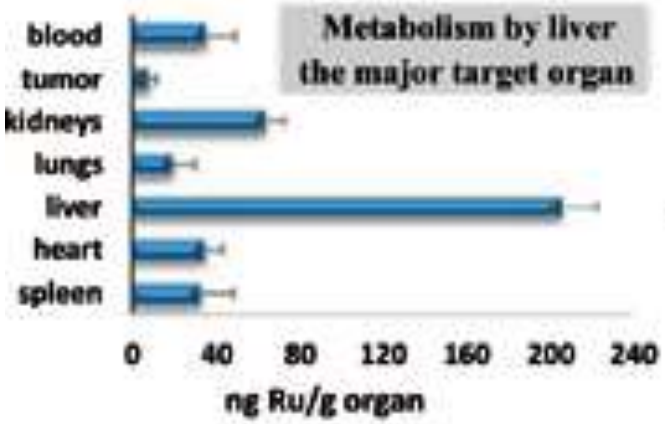
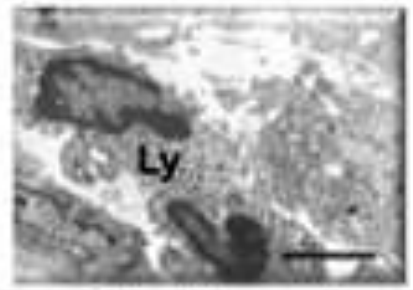


Xenograft Prostate Cancer Model

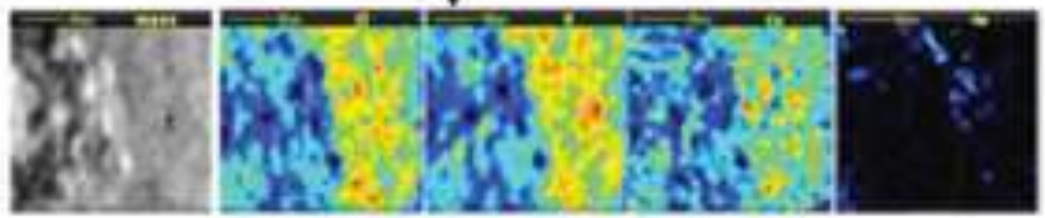


Metabolism by liver
the major target organ

Altered leukocytes
Lower nephrotoxicity



Morphology and
ultrastructural alterations:
Lymphocytes infiltration in
tumor



High Cl, K, and Ca associated with necrosis
Fe distribution associated with vascularization and
leukocyte infiltration

- The endocrine system is an important integrating system of the body.
- The various endocrine glands influence the following major physiological functions:

❖ maintenance of homeostatis - provides an optimum environment for the basic biochemical reactions of the body

❖ regulation of growth maturation

❖ reactions to exogenous stimuli (stress, starvation and infection)

❖ regulation of reproductive processes.

- Finely-regulated hormonal mechanisms make the endocrine system sensitive to toxic effects of exogenous compounds.
- These effects can interfere with the action, biosynthesis and release of hormones and result in alterations in hormonal target cells.

IN VIVO STUDIES

Clinical observations

- The choice of a suitable blood sampling method is of great importance if reliable measurements of hormones are to be obtained.

- In general, the effects of chemicals on the non-reproductive endocrine organs are not easily observed clinically.

- Gonadal dysfunction can be easily detected in reproductive studies, growth depression or changes in protein or carbohydrate metabolism ascribable to impaired pituitary, thyroid or pancreatic function can also result from other, non-endocrinal effects.

- Weight changes and histopathological findings are frequently indications of impairment of the endocrine system.

- Immunocytochemical techniques can be used to localize specific hormone producing cells. It has great advantage for many antigens to be demonstrated in paraffin sections of formalin fixed tissue.

- Sometimes, pre-treatment with proteolytic enzymes such as trypsin is necessary to unmask antigenic sites.

- Recent advances in immunocytochemistry allow the localization of antigens in plastic-embedded material both in light-microscopic and immunoelectron-microscopic techniques

- A recent and very interesting development in the fields of immunochemistry involves the use of monoclonal antibodies.

- Their defined specificity for tissue compounds enables them to be used in double staining procedures for the simultaneous localization of antigens on membrane structures or intracellular components.

Biochemistry of tissue and biological fluids

- Radioimmunoassay appears to be the most appropriate technique for the determination of hormones in body fluids and tissue homogenates.
- The use of many commercial clinical radio-immunassay kits for steroids and thyroid hormones are done in present time.
- Serum or plasma can be used in RIA methods and are preferred because of coagulation that occurs during thawing of frozen plasma samples.
- In principle, RIA can also be performed with urine although realizes that steroids are present in urine as conjugated glucuronides or sulphates.

Function tests

- These are valuable tool in assessing the endocrine toxicity of chemical compounds. Due to the complexity of the endocrine system, there is no single overall function test, but the various endocrine organs have their own specific tests based upon the uptake of radioisotopes or their reaction on 'externally' added hormonal stimuli.

- For the anterior pituitary, various tropic-hormone producing cells can be examined by stimulation with hypothalamic-releasing factors.

- Luteinizing hormone-releasing hormone also called luliberin, the release of LH and FSH can be determined in serum using RIA.

- In a similar manner, TRH or thyroliberin induces the secretion of TSH and prolactin into the serum, and corticotropic-releasing homone (corticoliberin) affects the ACTH producing cells.

- Following the administration of ACTH, the concentration of corticosterone can be determined in the serum as a measure of adrenal cortex function.

- For the study of pancreatic function, administration of glucose by the measurement of immuno-reactive insulin (IRI) is most appropriate.

IN VITRO TESTING

Thyroid

- The synthesis and secretion of thyroxin (T4) and triiodothyronine (T3) involves several steps that can be influenced by exposure to toxins. Dietary iodine is taken up as iodide by the thyroid in response to the binding of thyrotrophin (TSH) to cell membranes. T4 and T3 are formed by the coupling of iodotyrosyl residues within the thyroglobulin molecule and secretion of T4 and T3 occurs following proteolytic cleavage of these hormones from thyroglobulin.

- The oxidation and coupling reactions that lead to formation of T3 and T4 are inhibited by substances such as propylthiouracil and methylmercaptoimidazole. These compounds inhibit thyroid peroxidase, a membrane-bound haemoprotein which catalyses both oxidation and coupling reactions in thyroid tissue.

Adrenal gland

- The adrenal cortex is responsible for the synthesis and secretion of glucocorticoid which regulate many important functions of the body. The pituitary hormone adrenocorticotropin binds to membrane receptors on adreanl cortical cells which activate the adenylate cyclase systems and ultimately stimulate the synthesis of glucocorticoid hormones.

- The biosynthetic pathway for these hormones involves many enzymatic steps and utilizes cholesterol as the primary precursor.

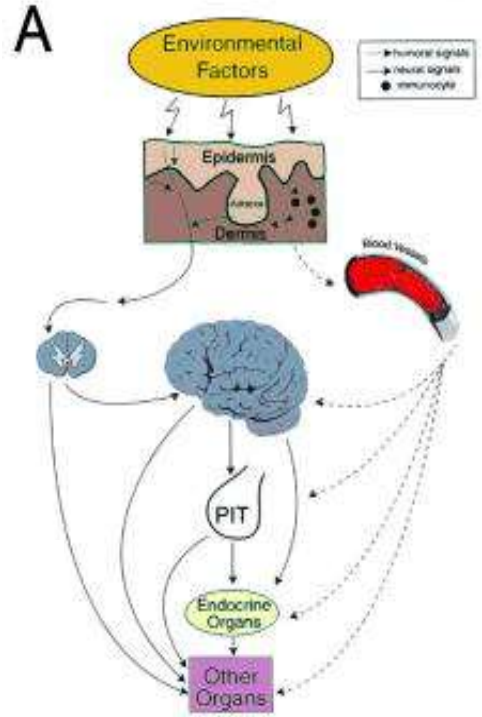
- Cholesterol is accumulated in the adrenal cortex by the endocytosis of lipoproteins from the blood under the control of ACTH.

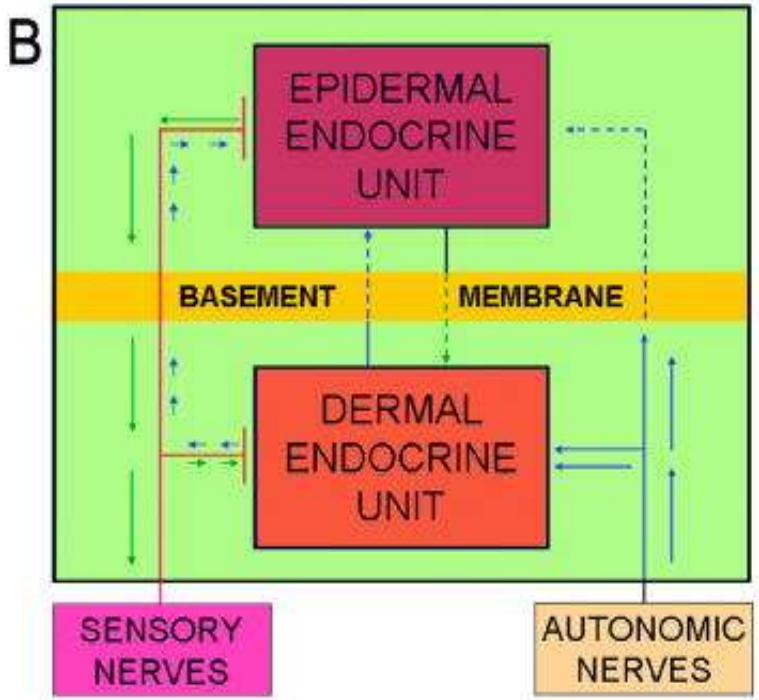
- Aldosterone is also synthesized by the adrenal cortex. However, its synthesis is primarily controlled by plasma sodium levels and angiotensin Il. The various control functions (such as receptors, enzyme activation and synthesis, pituitary secretion of ACTH, angiotensin Il production and action of adrenal steroids on target cells) present a complex array of possible points at which toxins could interfere with proper adrenal function.

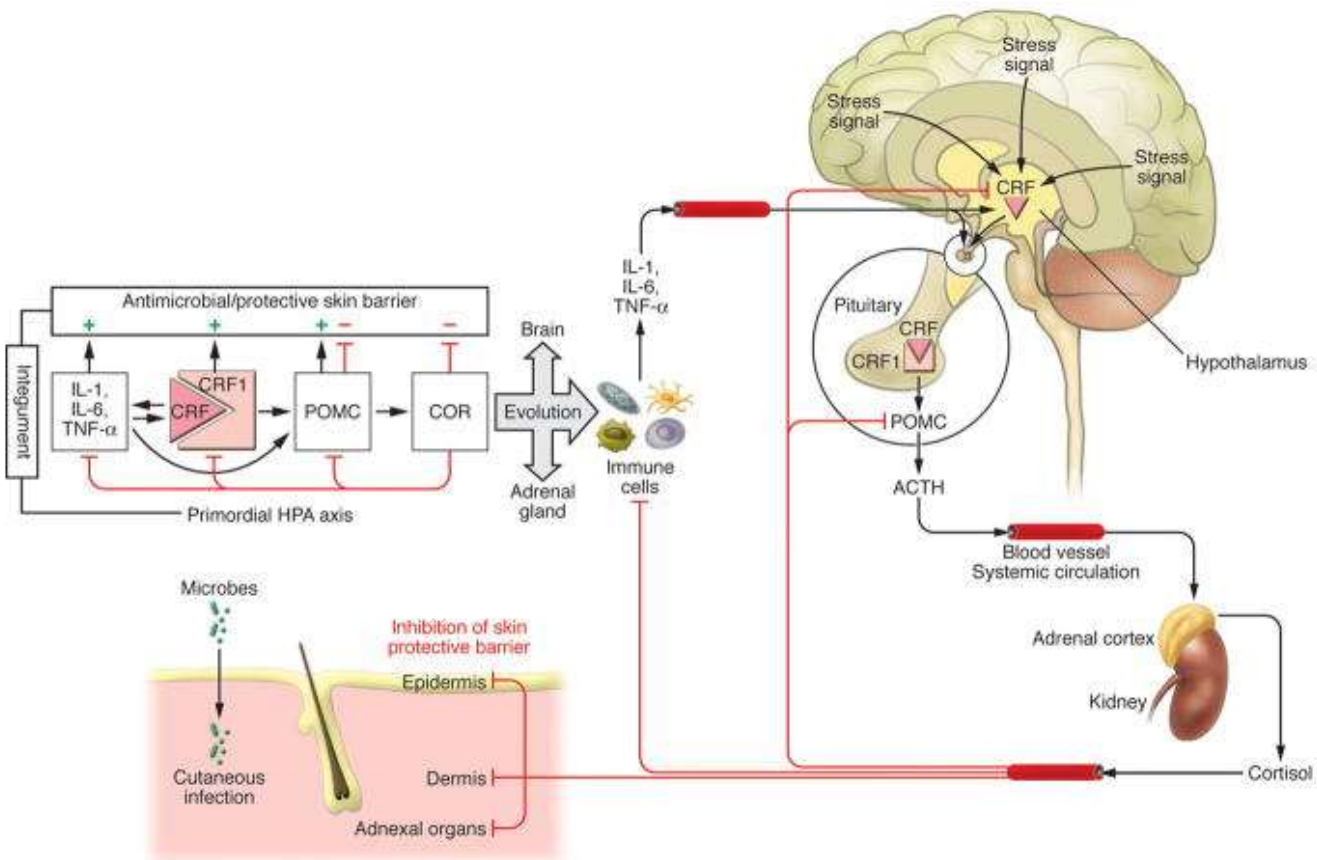
- The adrenal gland synthesizes cholesterol from acetate, it also acquires cholesterol by endocytosis of low density lipoprotein (LDL) from the blood. The influence of toxicants transferred by means of LDL-endocytosis on the activity of COA reductase (the rate limiting enzyme in cholesterol synthesis), acylCOA : cholesterol acyltransferase and synthesis of LDL receptors could be examined in adrenal cells or in cultured fibroblasts

Biosynthesis of adrenal steroids and enzyme inhibition

- The biosynthesis of adrenal steroids involves a complex pathway consisting of many enzymatic steps. The overall effect of a toxicant could be examined by adding 14C-cholesterol to adrenal cells or to glands in culture and measuring the production of 14C-corticosterone and/or 14C-aldosterone. One of the most important of these would be the conversion of cholesterol to pregnenolone which is performed by the desmolase enzyme complex; this is the rate-limiting step in steroid biosynthesis.







Homeostasis

Adrenal hormone action at the target cell

Glucocorticoids -

- The glucocorticoid hormones secreted by the adrenal cortex influence many important physiological functions. The concentration and activity of several liver enzymes are increased by glucocorticoid treatment. These increases appear to result from the binding of glucocorticoids to their respective receptor which stimulates the transcription of specific mRNAs that code for the synthesis of these enzymes. The binding of glucocorticoid receptor complexes to nuclear sites is closely correlated with the level of enzymatic stimulation. Thus occupancy of the receptor by hormone is closely coupled with response.

- The hormone also have an inhibitory or catabolic effect in many systems includes suppression of DNA synthesis, promotion of protein breakdown in muscle, suppression of immunological and inflammatory responses, and inhibition of cell proliferation in lymphoid, fibroblastic, epithelial and bone cells.

- Glucocorticoid receptors are present in many tissues and several cell lines. Receptors are present in the cytosol and nuclei of homogenized cells or tissues and the binding parameters can be determined by saturation analysis Classical competitive inhibition curves are easily performed and provide a rapid method for the determination of relative binding affinity. The binding of a chemical to the glucocorticoid receptor may interfere with nuclear accumulation of the hormone receptor complex. This possibility can be tested by measuring the nuclear accumulation of receptor following exposure of cells or tissues to both toxin and 3Hl-glucocorticoid. However, it would require analysis of the response parameters in order to establish whether the chemical is acting in an agonistic or an antagonistic manner.

Mineralocorticoids –

Aldosterone, regulate electrolyte balance in the kidney, salivary glands, sweat glands and the GI-tract. It augments the transport of sodium across epithelia by stimulating the synthesis of proteins that are involved in increasing the permeability of the apical membrane to sodium and the energy metabolism of the cell.

The receptors are present in target organs for mineralocorticoids in the kidney. These receptors form activated nuclear bound complexes in a fashion similar to those of other steroids. The mechanism by which aldosterone controls sodium transport probably involves the synthesis of proteins involved in the function of the sodium channel and energy production (ATP).

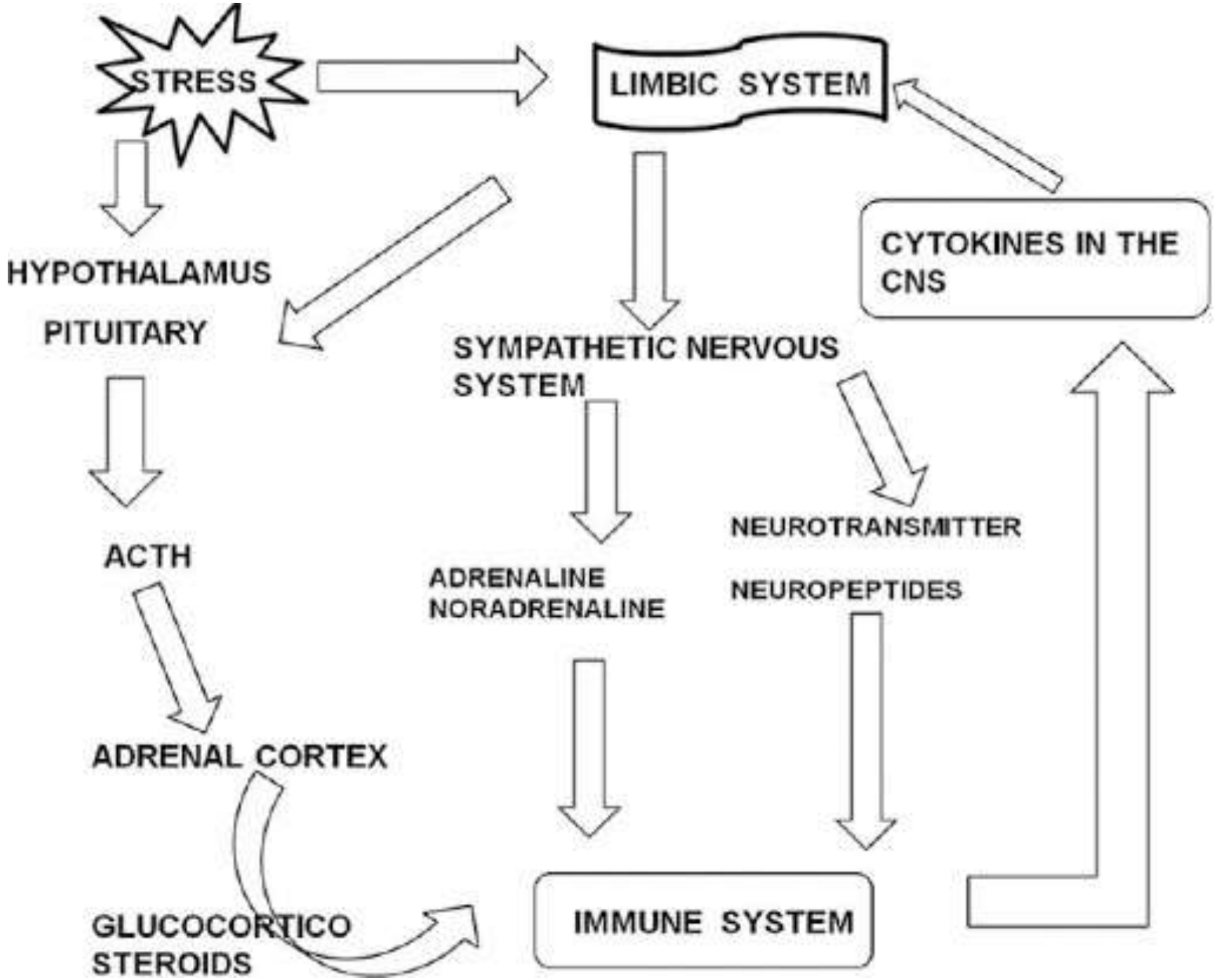
The increased ATP acts as an energy source for the sodium pump and also may increase the number of sodium pumps. Aldosterone also stimulates phospholipase activity, fatty acid synthesis and acyltransferase activity. All of these actions are probably involved in altering the membrane functions in the renal cell.

Hypothalamic—Pituitary system

- The hypothalamus produces releasing hormones that stimulate the secretion of pituitary hormones which control many other endocrine gland functions.

- The plasma membrane of anterior pituitary cells contains specific receptors for the various releasing hormones produced by hypothalamic neurones. These hormones include: corticotropin releasing hormone, which stimulates secretion of ACTH; thyrotropin releasing hormone, which stimulates secretion of TSH and growth hormone releasing hormone which stimulates the secretion of somatotropin.

- The binding of each of the releasing hormones is important for the production of pituitary hormones and thus could be an important point at which toxic substances could interfere with pituitary function.


STRESS
LIMBIC SYSTEM
HYPOTHALAMUS
PITUITARY
CYTOKINES IN THE CNS
SYMPATHETIC NERVOUS SYSTEM
ACTH
ADRENALINE
NORADRENALINE
NEUROTRANSMITTER
NEUROPEPTIDES
ADRENAL CORTEX
GLUCOCORTICO STEROIDS
IMMUNE SYSTEM

STRATEGY

Due to the complex nature of the endocrine system, no single test exists that can provide all the data that are necessary to assess the potency of compounds that may disturb endocrine functions. In vivo function tests of the various endocrine organs are indicated when the potential for chemicals to affect the endocrine system is established by:

- change in weight of the endocrine organs
- morphological alterations, detected with conventional as well as immunocytochemical staining
- changes in circulating hormone concentrations.

In vitro tests are potentially useful in order to ascertain:

- mechanisms of action (e.g. receptor binding studies, determination of enzymatic activity, release tests)
- the relative potency of chemical compounds that interfere with endocrine function.