

## BIOCHEMICAL RESPONSE OF THE SYMPATHO-ADRENAL AXIS

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### Introduction

Stress is a complex condition that frequently impacts the health and well being of both animals and man. Much of our knowledge about the response of the sympatho-adrenal axis is based on the classic work of Cannon (1) and Selye (2). Discharge of the sympatho-adrenal axis has been characterized as that which functions to prepare the body for "fight or flight". This response is generally thought to be rapid and to affect most of the organ systems in the body. However, additional research in this area has demonstrated that sympatho-adrenal discharge is regulated by mechanisms that can also produce long-term changes at the cellular level. The purpose of this communication is to review recently obtained knowledge about the response of the sympatho-adrenal axis to stress and its relationship to other stress responsive systems. For a more thorough discussion of the responses to stress situations, the reader should see references (3,4).

Activity of the sympathetic nervous system is modulated by a variety of stressors including heat, cold, immobilization, pain, psychological factors and starvation. The response generated to these stressors is dependent upon the duration, intensity and frequency of the stressor. Intense stressors that are applied for a short duration (on the order of seconds to minutes) can be functionally defined as producing a condition of acute stress and this correlates with the "fight or flight response" described by Cannon (1). These same stressors applied at a lower intensity over a period of days to weeks produces a condition termed chronic stress.

### Regulation of Catecholamine Biosynthesis in the Adrenal Medulla

The biochemical pathway for the synthesis of epinephrine in the adrenal medulla is illustrated in figure 1. Norepinephrine is thought to be synthesized by a similar pathway in the sympathetic nerve terminal. Since the sympathetic nerve terminal lacks phenylethanolamine N-methyl transferase (PNMT), norepinephrine is the terminal stage of neurotransmitter synthesis.

The initial and rate limiting step in the synthesis of norepinephrine and epinephrine is the hydroxylation of tyrosine to dihydroxyphenylalanine (DOPA) which is catalyzed by tyrosine hydroxylase (TH) (5). As the rate limiting enzyme in this biochemical pathway, the activity of tyrosine hydroxylase is known to be regulated by several biochemical mechanisms including feedback inhibition (6), phosphorylation by cyclic AMP-dependent protein kinase (7), phosphorylation by calmodulin-dependent protein kinase (8) and phospholipid/calcium-dependent protein kinase (Kinase C) (9), rate of de novo protein synthesis (10) and a variety of drug treatments (11, 19). The activity of tyrosine hydroxylase appears to vary directly with the frequency of impulses through the sympathetic nerves and TH has been used as a marker enzyme for increased sympathetic activity.

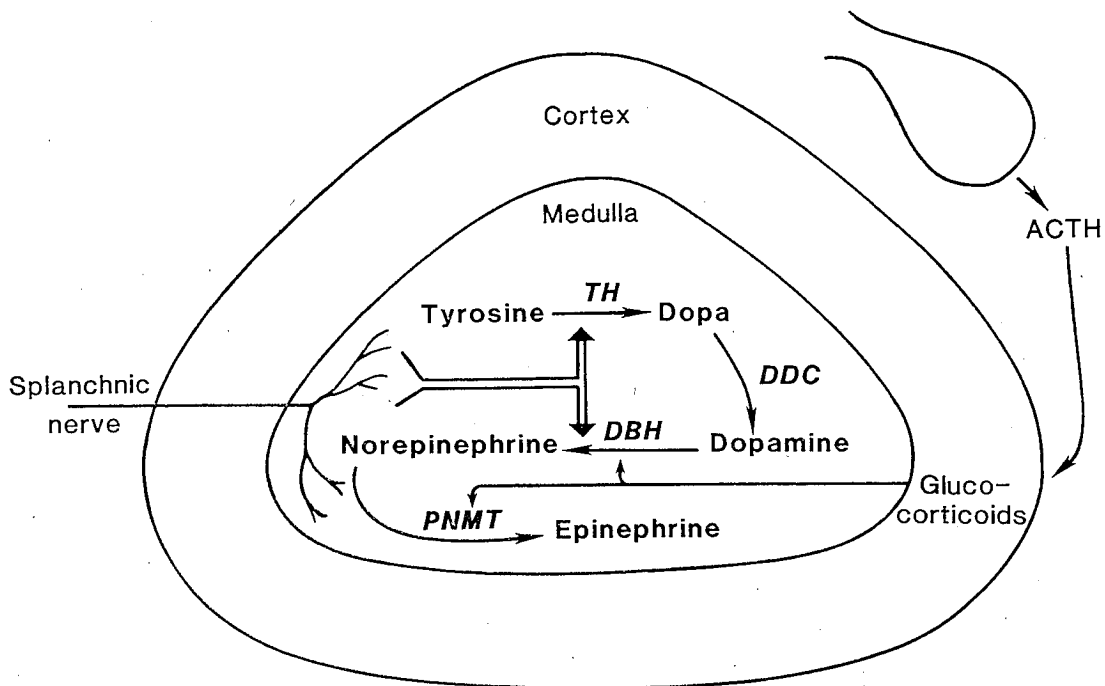


Figure 1. Pathway for the Biosynthesis of Epinephrine in the Adrenal Medulla.

As indicated in the schematic, splanchnic nerve activity is involved in regulation of cellular levels of TH and DBH. Glucocorticoids, released into the cortical-medullary portal system under the control of ACTH, modulate cellular levels of DBH and PNMT. TH = tyrosine hydroxylase, DDC = dopa decarboxylase, DBH = Dopamine  $\beta$ -hydroxylase, PNMT = phenylethanolamine N-methyl transferase.

### Systems Involved in the Stress Response

The systems known to be involved in mediating the stress response are diagrammed in figure 2. The cerebral cortex is essential to the organism for the perception of the stressor. The cortex sends nerve impulses to the hypothalamus and/or to the spinal cord. The hypothalamus controls the pituitary gland by well characterized specific "releasing factors". The pituitary gland responds to these factors releasing adrenocorticotrophic hormone (ACTH) which travels via the blood stream to the adrenal cortex to cause the release of cortisol (humans, dogs) or corticosterone (rats). Increase in nerve traffic down the spinal cord via the lateral funiculi causes an increased activity in the sympathetic preganglionic nerve fibers (13). The preganglionic nerve fibers innervate either the adrenal medulla or the postganglionic sympathetic nerve fibers. Increased nerve traffic results in release of the epinephrine from the adrenal gland or norepinephrine from sympathetic nerve terminals. The circulating level of norepinephrine is thought to reflect the activity of the sympathetic nerves and the plasma level of epinephrine has been used as an index of adrenal medullary function (4).

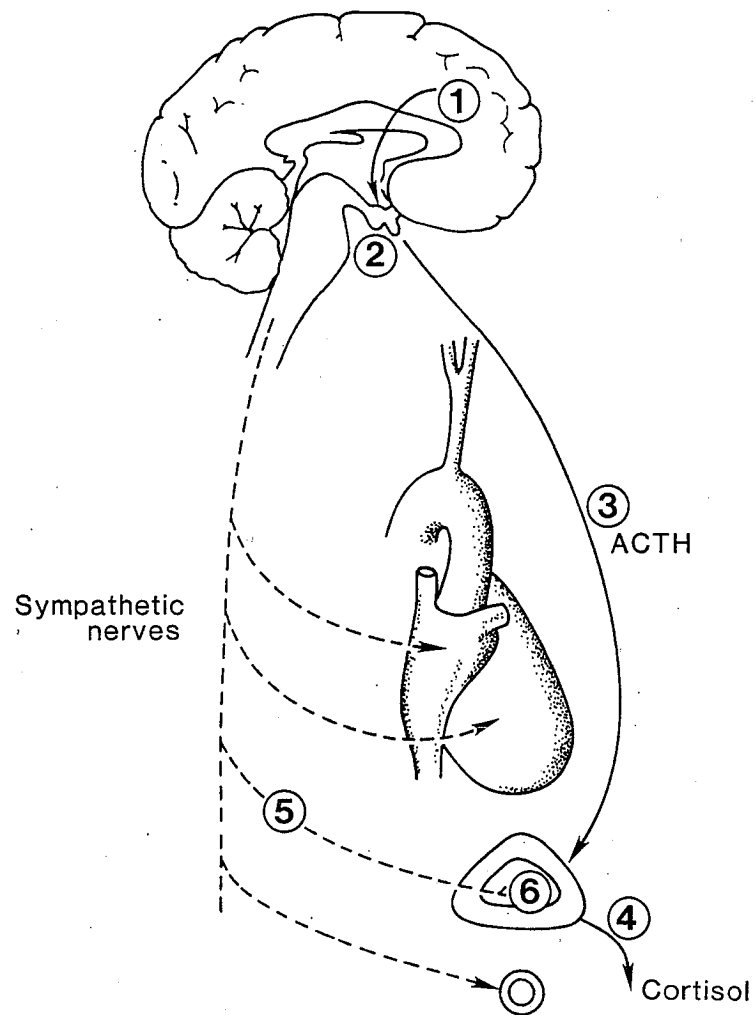


Figure 2. Systems Involved in Mediating the Stress Response.

Recognition of the stressor first occurs in the cerebral cortex (1). This information is then relayed to the hypothalamus/anterior pituitary (2) and results in increased release of ACTH (3) or down the spinal cord and sympathetic nerves (5). The increased nerve traffic is manifested as increased sympathetic tone of innervated organs or increased circulating catecholamines released from the adrenal medulla.

## Interaction of the Systems Mediating the Response to Stress

Perception of a stressor causes an alteration in both the sympatho-adrenal axis and the hypothalamic-adrenocortical axis. Increased neuronal activity in the sympathetic nervous system occurs during the stress response. This elevated activity increases the biosynthetic capability of the sympathetic nerve terminal and adrenal medulla as a result of activation of existing molecules of tyrosine hydroxylase (14). When the enzyme is assayed at substrate concentrations that are thought to be present in the adrenal medullary cells, TH activity will increase 4- to 8-fold as a result of this enzyme activation (15). The depolarization-dependent activation of tyrosine hydroxylase is thought to result from a direct phosphorylation of the enzyme, although the exact kinase involved has not yet been identified. Activation occurs rapidly, stimulation of adrenergic nerves will activate TH in as little as 15 seconds (15). Since newly synthesized norepinephrine is preferentially released during sympathetic nerve stimulation (16), this activation may play an important physiological role.

Repeated exposure to a stressor results in biochemical changes to the sympatho-adrenal axis that include a two- to four-fold increase in levels of tyrosine hydroxylase, dopamine  $\beta$ -hydroxylase (DBH) and phenylethanolamine N-methyl transferase (PNMT) (17, 18). The increase in adrenal medullary enzyme activity can be blocked by denervation or the presence of ganglionic blockers, demonstrating that trans-synaptic transmission is necessary for the induction of these enzymes (19). Induction of these enzymes results from increased synthesis of messenger RNA and de novo protein synthesis (9, 20). It takes about 24 hours before the changes in enzyme levels are apparent in the adrenal medulla. The levels of the biosynthetic enzymes remain elevated for up to two weeks following a period of chronic stress (9).

The glucocorticoids also play a role in the maintenance of normal adrenal medullary function. Maintenance PNMT levels require the presence of a relatively high concentration of glucocorticoids (21). These levels are achieved through an adrenocortical medullary portal system that insures that the medullary cells receive the blood that comes directly from the adrenal cortex. Animals that have been hypophysectomized will have very low levels of PNMT and reduced levels of DBH. These changes in enzyme levels can be reversed with the administration of either ACTH or very high levels of dexamethasone (22). Maintenance of DBH levels is also dependent upon an intact nerve supply. Denervation will result in a decrease in DBH. [On the other hand, nerve stimulation, which will induce TH, also results in increased in DBH levels (18).] Therefore, it appears that the level of DBH is regulated by at least two independent mechanisms.

The activity and concentration of the enzymes involved in biosynthesis of the catecholamines are clearly regulated by the previous experience of the animal. We know that living in stressful environments will cause increased levels of these enzymes as measured in vitro. However, more research is needed to evaluate the role that increased levels of these enzymes plays in regulating the responsiveness of the sympathetic nervous system to stress in the intact animal.

## Alterations in the Bioreactivity of the Sympatho-adrenal Axis

The mechanisms described above are involved in producing the physiological response to stress. The type of stressor, duration and intensity of the stress all play a part in determining the ultimate response to the stress. However, an important question remains; Can the response of the sympatho-adrenal axis be altered by previous experience or environmental situation? The majority of the research performed to date has involved the study of the stress response of single tissues and in response to individual stressors. Very little research has been performed which addresses this question, but the preliminary indications are that the bioreactivity of the sympatho-adrenal axis can be altered by previous experience. Recently, Kvetnansky and coworkers have demonstrated that rats exposed to chronic (long-term) stress will demonstrate enhanced sympatho-adrenal reactivity to novel acute stressors when compared with non-stressed controls. Circulating levels of epinephrine and norepinephrine increased nearly sixteen-fold and ten-fold respectively (23). The mechanism of the enhanced reactivity is an active area of investigation.

## Conclusion

Stress is a condition that affects most of the organs of the body. In spite of years of research, little is known about the exact mechanisms involved in the stress response. We know that conditions of stress will produce a weakening of the physiological defense mechanisms and enhance the occurrence of disease (24). We have identified many of the major organs involved in the stress response and their interactions. However, more research is needed to investigate the temporal relationship of stress to its physiological responses (e.g. Is a long-term mild stress more insidious than a short-term intense stress?).

## References

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Points raised during discussion

Question: Is the tyrosine hydroxylase (TH) induced in animals with the enhanced stress response?

Dr. Vulliet: Yes, in the animals that were examined the TH was induced. There is a reasonable correlation between the induction of TH and the enhanced bioreactivity of the sympathetic nervous system.

Question: May there be other explanations for the enhanced stress response, possibly increased storage capacity or other changes in the adrenal medulla?

Dr. Vulliet: We do not know yet. Now that we have the techniques we hope to be looking at the other possibilities.