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Adrenal Chromaffin Cells and Stress

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Summary. **This review deals with the function of the adrenal chromaffin cell under stress. Although the terminology of "stress" is rather confusing, effects of certain kinds of stress stimuli (emotional stress, physical stress etc.) on [1] the secretion of catecholamines from the adrenal medulla are reviewed first. In the next sections, discussion is focused on the effect of stress on [2] biosynthesis and [3] the reuptake of catecholamines. Stress effects on [4] enkephalin metabolism in the adrenal medulla and [5] the possible roles of neuropeptides in the medulla under stress are also discussed.**

In conclusion, stress increases the secretion of catecholamines from the adrenal medulla and consequently levels of peripheral catecholamines, due to enhanced catecholamines synthesis, and decreased reuptake and degradation. One possible role of neuropeptides under stress is to modulate catecholamines secretion from chromaffin cells.

Introduction

The investigation of catecholamines started when Oliver and Schäfer[1-4] found that an injection of the extract of the adrenal gland caused a dramatic rise in blood pressure. In 1898, John Abel announced that he had isolated the active principle in the adrenal gland, naming it "epinephrine".[5,6] Otto von Fürth, who had been working on the active principle of the adrenal gland,[7,8] questioned Abel's result and presented a different body which he called "suprarenin".[9] However, what they called "epinephrine" or "suprarenin" were not pure bodies but mixtures. Jokichi Takamine succeeded in obtaining a stable crystalline body of constant composition of the adrenal gland, naming it "adrenalin".[10,11]

After the isolation of adrenaline, a second active principle for the adrenal medulla was predicted by many investigators including Szent-Györgi[12] or Yasutaro Satake.[13] However, their speculations that their results could not be explained by a single active principle in the medulla were later revealed to be due to the assay method they emplyed. Finally, in 1949, von Euler reported the isolation of the second active principle of the adrenal medulla and identified it as "noradrenaline".[14] It was identical with "sympathin"[15] estimated by Cannon as the active principle in the sympathetic nerve endings. Von Euler also found that there was an increased excretion of noradrenaline in urine after stress.[16,17]

In 1924, Walter Cannon[15] examined the effect of physiological changes on movements of the dog stomach. He noted that when the animal was submitted to stressful situations, there was a marked suppression in the movements of the stomach. Later he found that it was the sympathetic nerves that influenced the movements of the stomach under stress. He partially identified the humoral factor released from sympathetic nerves and called it "sympathin".

Hans Selye[18] found that under stress there was an enlargement of the adrenal gland. He was the first to introduce the concept of stress to scientists when he proposed the general adaptation syndrome. According to his syndrome, there are three phases to stress: (1) The alarm reaction, (2) adaptation, and (3) finally, after constant stress, exhaustion.

These predecessors proposed that the adrenal gland—cortical steroids and medullary catecholamines—play important roles under stressful conditions. In this review, discussion is focused mainly on the adrenal medullary function under stress.

The functions of the adrenal medullary chromaffin cells are biosynthesis, release and inactivation

This paper is dedicated to the late Dr. Sinosaburo Suzuki.

(reuptake and degradation) of catecholamines. Recent advances suggest that adrenal chromaffin cells represent not only the factory of catecholamines but also that of certain neuropeptide such as enkephalins. neuropeptide Y, vasoactive intestinal polypeptide (VIP), substance P, somatostatin and calcitonin gene-related polypeptide (CGRP). We shall discuss stress effects on catecholamines, those on enkephlin and other neuropeptides being covered later.

[1] Secretion of Catecholamines from Adrenal Medulla by Stress

The release of catecholamines from the adrenal medulla is interpreted as "stimulus-secretion coupling", proposed by Douglas.[19] Action potentials arriving at the preganglionic nerve terminal induce acetylcholine release. Acetylcholine binds to nicotinic acetylcholine receptors on the chromaffin cell membrane and elicits depolarization. This depolarization opens up a voltage dependent Ca-channel: Ca influx. Increased intracellular calcium then releases chromaffin granules from the cytoskelton and helps them move and fuse to the cell membrane. The contents of the chromaffin granule are released by exocytosis.[20]

Since the beginning of this century, although such an intracellular process was unknown, many investigators paid attention to the release of catecholamines induced by various kinds of stressful stimuli. Stress was known to activate both the sympathetic adrenomedullary system and pituitary adrenocortical system. In 1911, Walter Cannon formulated an emergency reaction, a stress theory, of adrenomedullary activity.[15] Selye's stress theory developed in the 1940's was based on the activation of the pituitary adrenocortical system.[18]

In this section on the secretion of catecholamines from the adrenal medulla under stress, the contributions of Yasutaro Satake and his collaborates must be included. Of over 300 papers—including the experiments he guided during his 30 years of Tohoku University—about 84% dealt with the adrenal medullary function under stress or the effects of drugs on it. He summarized his and his collaborates' work in a monograph.[21]

Stimulation of adrenal catecholamine discharge

Cannon and de la Paz first showed that emotional excitement in an animal leads to a general stimulation of the sympathetic nervous system.[22] Emotional stress increased adrenaline release from the adrenal medulla.[17,23]

The response to cold is infused with emotional stress. Klepping found not only a marked increase in total secretion into the adrenal vein blood, but an increase in the proportion of adrenaline.[24] Wada et al., under Satake's guidance, showed that, even after adrenalectomy, exposure to cold produced a considerable increase in the rate, suggesting that sympathetic nerve activity plays an important part in the response.[25] Saito, whose experiment was carried out in Satake's lab, was the first to observe that adrenal medullary secretion occurred in response to surface burns.[26]

Metabolic acidosis[27] as well as meatabolic alkalosis[28] has been demonstrated to activate adrenomedullary secretion through the central nervous system.

Physical exercise is accompanied by an increased excretion of catecholamines in the urine.[23,29-31] Adrenaline content is decreased in the medulla after exercise.[32] The relative amount of adrenaline is not changed by exercise. Von Euler[16] concluded that the source of increased urinary catecholamines during exercise was both the adrenal medulla and sympathetic nerves.

Asphyxia leads to the secretion of adrenaline from the adrenal glands. Cutting the splanchnic nerves prevents the response to anoxia.[33] In perfused adrenals, with the splanchnic nerves cut, a period of anoxia causes a discharge of adrenaline.[34] Asphyxia represents a combination of stimuli to the central structures governing the sympathetic nervous system and directly to the adrenal chromaffin cells. The neurogenic adjustments always precede and overshadow those of catecholamines from the adrenal medulla.[35]

It is generally accepted that the role of adrenal catecholamines is not significant in the maintenace of normal blood pressure. However, there is no doubt that the adrenal medulla responds to abnormally low blood pressure. The induced hypotension is accompanied by an increased secretion of catecholamines into the adrenal vein biood. In hemorrahagic hypotension, there is an increase in the secretion of catechollamines which is proportional to the decrease in blood volume.[36,37] Niijima[38] reported that hemorrahagic hypotension induces increased sympathetic outflow to the adrenal gland, and interpreted this as a baroreceptor reflex. Carotid occlusion causes an increase in adrenal catecholamine discharge.[39]

Hypoglycemia, such as that following an overdose of insulin, was shown to cause the secretion of cate-

cholamine from the adrenal medulla.[15] During insulin hypoglycemia, the adrenal medulla is depleted of adrenaline and the proportion of noradrenaline increased.[40] The depletion continues even after the blood sugar has returned to normal levels.[41] Ikeda found that, in dogs with spinal transection, the effect of hypoglycemia was still observed; whereas after splanchnectomy, the response was abolished.[45] Niijima[42,43] showed that insulin hypoglycemia increases sympathetic efferent activity to the adrenal gland while glucose injection inhibits the activity. Glucose-sensitive neurons in the hypothalamus are involved in this reflex.[44]

Some naturally occurring substances, i.e. bradykinin, angiotensin II or histamine, are known to elicit adrenal catecholamine secretion by directly stimuiating the chromaffin cells. These substances are released on stress.

The proportion of adrenaline and noradrenaline differs according to the kind of stress: there is a selective release of the two catecholamine. Stimulation of the different areas of the hypothalamus elicits different proportion of adrenaline and noradrenaline.[46,47] There are two kinds of nerve fibers in the sympathetic branches to the adrenal medulla, their activity being reflexly inhibited by alpha or beta adrenergic stimulants, respectively.[48,49] There also are two kinds of chromaffin cells in the medulla: adrenaline-storing cells and noradrenaline-storing cells.[50-52] Thus the two catecholamines in the medulla are considered to be controlled by indepedent pathways. Integration could be carried out in the central nervous system.

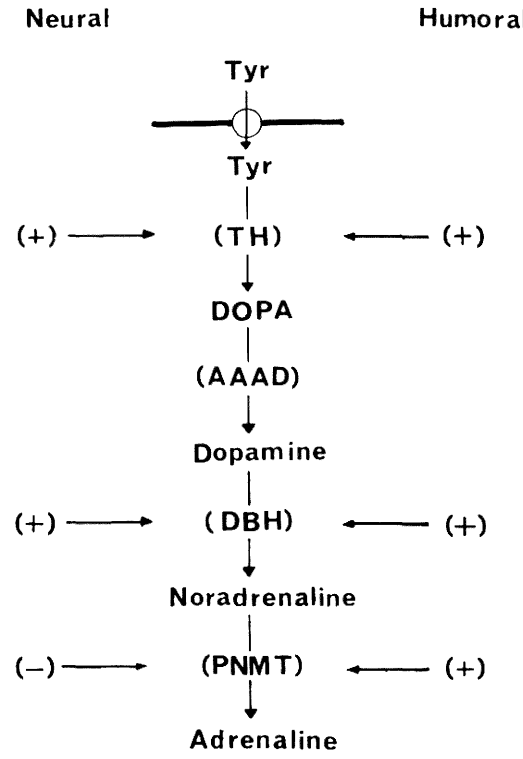

Fig. 1. Neural and humoral influence on catecholamine synthesizing enzymes. Tyr: tyrosine, TH: tyrosine hydroxylase, DOPA: 3, 4-dihydroxy phenylalanine, AAAD: aromatic L-amino acid decarboxylase, DBH: dopamine β-hydroxylase, PNMT: phenylethanolamine N-methyl transferase.

[2] Stress Effects on Catecholamine Synthesizing Enzymes in the Adrenal Medulla

The scheme of catecholamine synthesis was suggested by Blaschko in 1939.[53] This metabolic pathway is illustrated in Fig. 1. The first step in catecholamine synthesis is the uptake of the precursor, tyrosine, into adrenal chromaffin cells. The transport of tyrosine is a carrier-mediated facilitated diffusion for neutral amino acids.[54] The oxidation of tyrosine to 3, 4-dihydroxyphenylalanine (DOPA) is catalyzed by tyrosine hydroxylase (TH). TH is the rate-limiting enzyme of catecholamine synthesis.[55] Dopa decarboxylase (aromatic L-amino acid decarboxylase) was the first enzyme to be described in the noradrenaline synthesis.[56] It is widely distributed in animal tissues. This enzyme catalyzes the decarboxylation of DOPA to 3,4-dihydroxy phenylethylamine (dopamine). It was originally called DOPA decarboxylase (DDC); however, aromatic L-amino acid decarboxylase (AAAD) is a more accurate name. The third step in catecholamine synthesis is the beta-hydroxylation of dopamine to form noradrenaline, which is catalyzed by dopamine β-hydroxylase (DBH). This enzyme is localized in chromaffin granules and released concomitantly with other constituents in the granules: noradrenaline, ATP, chromogranins etc. In sympathetic post-ganglionic neurons and adrenal noradrenaline-storing cells (NA cells), it is the final step. The last step in the synthesis of adrenaline in adrenaline-storing cells (A cells) or adrenaline neurons in the central nervous system is the N-methylation of noradrenaline catalyzed by phenylethanolamine N-methyl transferase (PNMT).

The adrenal medulla was long held to be controlled

by nerve impulses carried to the medulla by the preganglinonic sympathetic nerves. Catecholamine biosynthesis in the adrenal medulla is influenced by nerve impulses to the organ. Chronic stimulation of the splanchnic nerves and chronic stimulation of the adrenal gland by pharmacological means are associated with increased levels of the enzymes TH and dopamine β-hydroxylase (DBH).[57-60] The depletion of catecholamines in the periphery and central nervous system by reserpine results in an increased TH and DBH activity.[61-63] This increase in TH and DBH activity has been shown to occur as a result of the increased induction of their mRNA.[64-66]

In mammals, adrernal chromaffin cells are present in the interior region of the adrenal gland, surrounded by cortical tissures. Adrenal arterial blood is considered to supply first cortical tissues and then proceed on to the medullary sinus and bath adrenal chromaffin cells, forming a kind of protal system.[67,68] The adrenal medulla is situated downstream from the cortex. Thus medullary blood contains highly concentrated cortical hormones. In 1966 Wurtman and Axelrod reported that glucocorticoids, which are secreted from the adrenal cortex, enhanced the activity of PNMT in the adrenal medulla by a new synthesis of enzyme protein.[69] This was the first study showing that the biosynthesis of adrenal catecholamines is regulated by the humoral pathway. Activities of TH,[70] DBH[71,72] and PNMT[73] decrease following hypophysectomy. To restore the adrenal PNMT level to normal, a moderate dose of ACTH is enough, but a considerably large dose of dexamethasone is required.[73]

These changes in enzymatic activities by glucocorticoids are accompanied by concomitant alterrations in the relative abundance and cell-free translation activity of TH mRNA.[74] The maintenance of PNMT mRNA level is dependent on corticosterone levels.[75,76]

Actue single immobilization slightly increases activities of TH and PMNT, while as the stress is repeated, the activities of TH,[77] DBH[78] and PNMT[79] are markedly increased. Adrenal denervation prevents the increased activity of TH and DBH but not PNMT.[79] In hypophysectomized rats, the activity of TH and DBH is slightly increased by repeated, immobilization.[79] Prolonged activation of catecholaminergic cells during exposure to cold leads to adaptative changes in catecholamine synthesis. These changes include an increase in the mass of biological activity of the mRNA.[64,80,81] In the adaptive phase, increased activities of the sympathetic nervous and pituitary-adrenocortical system ehance the activity of the catecholamine synthesizing enzymes to produce enough catecholamines to adapt to the stress.

[3] Uptake of Catecholamines under Stress

Adrenal chromaffin cells have an ability to accumulate circulating catecholamines against a concentration gradient.[82-86] The uptake system is generally similar to that of sympathetic nerve endings but has a slight difference: there is no stereo-specificity. It is also apparently different from that of chromaffin granules. The affinity of catecholamines to this amine uptake system can be shown as follows: dopamine > noradrenaline > adrenaline.[86] It is an energy dependent carrier mediated catecholamine transport. The physiological significance of the uptake system is to inactivate released catecholamines and reutilize them. Thus, the uptake is believed to be inhibited when catecholamines are released. The uptake system is under the dual control of the pituitary adrenocortical and sympathetic nervous systems (Fig. 2).

Humoral Control of Amine Uptake

In autoradiographic studies, subcortical chromaffin cells incorporated more [^{3}H]dopamine than those

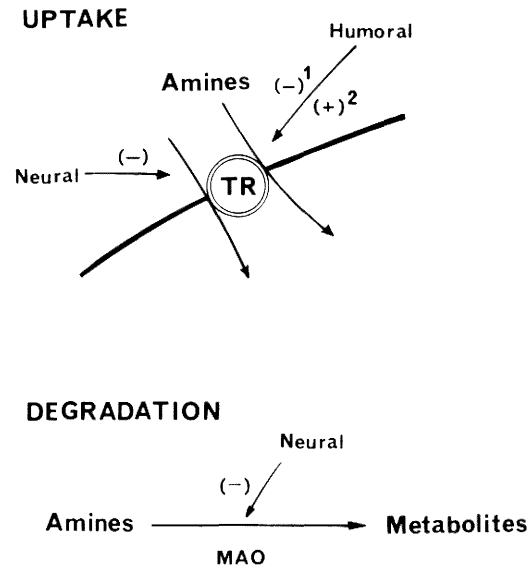

Fig. 2. Neural and humoral control of amine uptake and degradation in adrenal chromaffin cells. TR: Amine transporter, 1: If pituitary-adrenocortical activity disappears, uptake is suppressed, while 2: a high dose of corticosterone inhibits the uptake. MAO: monoamine oxidase.

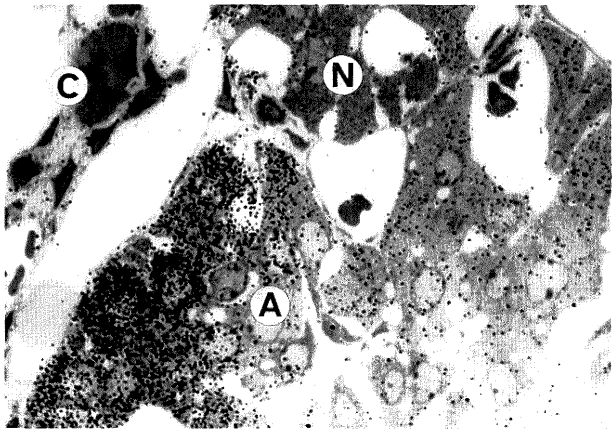

Fig. 3. Light microscopic autoradiograms of the mouse adrenal medulla at 30 min after the injection of [^{3}H]-dopamine. An accumulation of autoradiographic silver grains is seen on the chromaffin cells situated near the cortico-medullary junction. A: Adrenaline-storing cells, N: noradrenaline-storing cells, C: cortical cells.

situated in the center of the medulla[83] (Fig. 3). This suggested that the amine uptake system works under the influence of adrenocortical hormones since the adrenal artery first supplies the adrenal cortex, then the medulla.[87] The medullary blood contains highly concentrated cortical hormones. In hypophysectomized mice, [^{3}H]dopamine uptake is suppressed; the suppression is restored by the treatment of the hypophysectomized animals with a replacement dose of ACTH or a large dose of corticosterone.[88,89] Immobilization caused the inhibition of [^{3}H]dopamine uptake and accelerated release of incorporated [^{3}H] dopamine-derived radioactivity. In immobilized animals, both the pituitary-adrenocortical system and sympathetic nervous systems are activated. Adrenal denervation itself resulted in an enhancement of dopamine uptake, but stress had no effect on the uptake of dopamine in devervated chromaffin cells. Thus excess cortical hormones seemed not to have any influence on this uptake system. However, in isolated chromaffin cells, corticosterone and dexamethasone caused an inhibition of [^{3}H]dopamine uptake.[90] ACTH had no direct effect on the uptake of dopamine. These results may lead to the conclusion that cortical steroids are necessary to maintain basal amine uptake activity, but that excess cortical steroids rather inhibit the uptake.

Neural Control of amine uptake

In autoradiographic studies, adrenal denervation caused an enhancement of [^{3}H]dopamine uptake.[91,92] An acute, single immobilization was applied to the mouse with unilateral adrenal denervation to examine the effect of increased sympathetic outflow. The uptake of dopamine was remarkably inhibitied by the immobilization and the inhibition was prevented by adrenal denervation.[92,93] Increased sympathetic activity, then, inhibits the uptake of dopamine in chromaffin cells, as has been supported by an *in vitro* study. In isolated chromaffin cells of the guinea pig, acetylcholine or high potassium concentration in the medium inhibits the uptake of [^{3}H]noradrenaline.[86] Some other naturally occurring substances such as bradykinin or VIP also inhibit the uptake of dopamine in isolated chromaffin cells of the mouse.[94]

These results may indicate that, under acute stress, both increased neural activity and the pituitary adrenocortical system inhibit the uptake of catecholamines into adrenal chromaffin cells. Under stress, catecholamines are needed to bear the condition. The inhibition of catecholamin uptake—one of the inactivating processes—may help to prolong the biological action of catecholamines.

Repeated immobilization brought a completely reversed response. On the 8th immobilization, the uptake of [^{3}H]dopamine was not suppressed but rather enhanced.[95] As the stress continued, catecholamine synthesis was enhanced by the increasing biosynthesis of enzymes for catecholamine synthesis. The increased uptake may be due to enhanced inactivating process for excess production of catecholamines. The uptake of catecholamines into sympathetic nerve endings under stress remains undetermined.

Another inactivation process for catecholamines, degradation by enzymes, is also influenced by stress. Inhibition of monoamine oxidase by phenelzine is reduced by stress,[96] or cold stress reduces its activity.[97]

The inactivation processes of catecholamines is inhibited by stress, presumably to extend the activity of catecholamines released by stress.

[4] Stress Effect on Enkephalin in Adrenal Chromaffin Cells

Since Schultzberg et al.[98] first demonstrated the existence of enkephalin-like immunoreactivity in adrenal medullary cells, great interest has been focused on enkephalins in adrenal chromaffin cells. Enkephalins coexist with catecholamines in chromaffin granules and are released concomitantly from chromaffin cells on nicotinic stimulation.[99] Released enkephalins suppress the release of catecholamines from the adrenal medulla.[100] Nicotinic

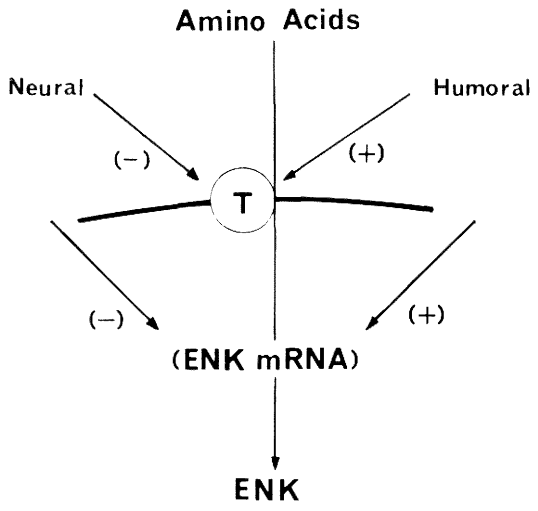

Fig. 4. Neural and humoral influences on enkephalin metabolism in adrenal chromaffin cell. T: Neutral amino acid transporter on chromaffin cell membrane, ENK: Enkephalins.

receptor stimulation activates enkephalin biosynthesis in adrenal chromaffin cells.[101] Adrenal denervation increases the content of enkephalin by increasing proenkephalin mRNA in the adrenal gland.[102] Dexamethasone increases the cell contents of proenkephalin mRNA.[103] The level of enkephalins in the rat adrenal medulla is regulated by glucocorticoids.[104] Pharmacological destruction of the adrenal cortex decreases the level of enkephalin in the medulla.[105] These reports suggest that the metabolism of certain peptide hormones such as enkephalins could be affected by stress. Recently we have revealed that the transport of [^{3}H]leucine, which is incorporated into enkephalins or other constituents in adrenal chromaffin cells, is suppressed by hypophysectomy but is increased by immobilization stress.[106] This may indicate that not only enkephalin biosynthesis but also incorporation of extracellular amino acids is neurally and humorally controlled under stress (Fig. 4).

[5] Neuropeptides and Adrenal Chromaffin Cells

Recent immunohistochemical studies have shown the coexistence of certain biogenic amines and neuropeptides in paranuerons[107] and neurons. Many kinds of neuropeptides have been demonstrated in the adrenal chromaffin cells and nerve terminals in the medulla.

Vasoactive intestinal polypeptide (VIP),[108,110] substance P,[111] enkephalins,[112,114] neurotensin,[115,116] neuropeptide Y,[117,120] and somatostatin[116,121] are known to exist in the adrenal medulla. VIP,[110,122,123] enkephalins[121,124,125] and neuropeptide Y[119,120] are also found in nerve terminals in the medulla.

Levels of several neuropeptides including vasopressin, corticotropin-releasing hormone (CRH), neuropeptide Y, and enkephalin are regulated by glucocorticoid, by its controlling the level of mRNA coding.[103,126,129] Stress induced changes in the neuronal and humoral environment may alter the production of neuropeptides by changing the level of mRNA coding.

Of a number of neuropeptides localized in the medulla, only a few peptides are known for their physiological functions.

VIP elicits non-cholinergic Ca-dependent catecholamine secretion from chromaffin cells.[130,131] Substance P suppresses catecholamine secretion by acetylcholine.[132–133] Substance P has been shown to protect against nicotinic desensitization, this modulatory function of substance P likely important for maintaining catecholamine secretion during times of stress.[134,136] Somatostatin also inhibits nicotine-induced catecholamine secretion from guinea-pig adrenal chromaffin cells. Met-enkephalin, Leu-enkephalin, β-endorphin, dynorphin-13, Met-enkephalin-Arg-Phe and BAM-22P inhibit nicotin- and acetylcholine-induced catecholamine secretion from isolated bovine adrenal chromaffin cells.[137,144] One siginificant role of these endogenous opioid peptides may be to regulate catecholamine secretion under stress and provide analgegic effect when stress is accompanied with pain. However, the roles of these neuropeptides in the medulla under various kinds of stressful stimuli have yet to be fully investigated.

Conclusion

Due to its peculiar localization in the gland and embryological origin, the adrenal chromaffin cell function is controlled by both sympathetic nervous and pituitary-adrenocortical systems. Since stress activates both systems, the functions of the adrenal chromaffin cells—secretion, biosynthesis and inactivation (reuptake and degradation) of catecholamines—are neurally and humorally influenced by stress. Repeated stress increases catecholamine levels in

peripheral tissues, blood, urine and also in the brain, due to enhanced catecholamine synthesis, and suppressed degradation of catecholamines. As the stress is repeated, however, the uptake of catecholamines is rather enhanced, probably to incorporate excess catecholamines. The actual function of these increased catecholamine levels in repeatedly stressed animals is still to be elucidated. It also remains to be determined whether this phenomenon is favorable for the organism or not. In addition, recent progress has revealed that not only catecholamines but also neuropeptides in the adrenal medulla are under the control of both systems. Catecholamines and neuropeptides are involved in adaptive reactions to stress. Knowledge of the roles of these substances under stress is however, still insufficient.

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Addendum

Dr. Sinosaburo Suzuki, the author's grandfather, graduated from Kyoto Prefectural University of Medicine. When he was a student, professor Yasutaro Satake was professor of Physiology, so there is a possibility that he attended professor Satake's lecture. Professor Y. Satake was one of the first professors in the author's department. More than 60 years later, the author attended the lecture of professor Mei Satake, his son, at Niigata University's School of Medicine. In addition, the author's abiding interest for these ten years has been stress and the adrenal medulla. The author cannot help wondering about the existence of Karma.

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