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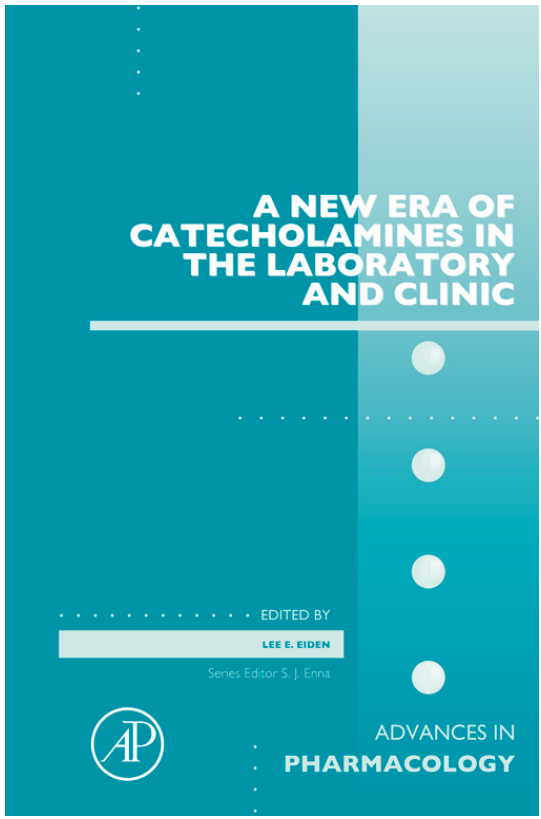

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CHAPTER SEVENTEEN

Stress-Triggered Changes in Peripheral Catecholaminergic Systems

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Contents

Abstract

The sympathetic nervous system not only regulates cardiovascular and metabolic responses to stress but also is altered by stress. The sympathoneural and sympathoadrenomedullary systems are modified by different metabolic pathways and have

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different responses to short- and to long-term stressors. Stress also induces nonneuronal catecholamine enzymes, primarily through corticosteroids. Catecholamine synthetic enzymes are induced by different pathways in response to short- and long-term acting stressors, like cold exposure or immobilization, and differently in the sympathetic ganglia and the adrenal medulla. However, a long-term exposure to one stressor can increase the response to a second, different stressor. Tyrosine hydroxylase gene transcription increases after only 5 min of immobilization through phosphorylation of CREB, but this response is short lived. However, repeated stress gives a longer-lived response utilizing transcription factors such as Egr-1 and Fra-2. Glucocorticoids and ACTH also induce sympathoneural enzymes leading to distinct patterns of short-term and long-lived activation of the sympathetic nervous system.

Nonneuronal phenylethanolamine *N*-methyltransferase (PNMT) develops early in the heart and then diminishes. However, intrinsic cardiac adrenergic cells remain and nonneuronal PNMT is present in many cells of the adult organism and increases in response to glucocorticoids. Both stress-induced and administered glucocorticoids induce fetal PNMT and hypertension.

Human stressors such as caring for an ill spouse or sleep apnea cause a persistent increase in blood norepinephrine, increased blood pressure, and downregulated catecholamine receptors. Hypertension is associated with a loss of slow-wave sleep, when sympathetic nerve activity is lowest. These findings indicate that stress-induced alteration of the sympathetic nervous system occurs in man as in experimental animals.

ABBREVIATIONS

DA dopamine
DBH dopamine-beta-hydroxylase
E epinephrine
HPA hypothalamic–pituitary–adrenocortical
ICA intrinsic cardiac adrenergic (cells)
NE norepinephrine
PNMT phenylethanolamine *N*-methyltransferase
TH tyrosine hydroxylase

1. INTRODUCTION

Stress accelerates cardiac output, respiration, catabolism, and blood flow in response to increased sympathetic nervous activity. These responses are normally transient and adaptive. However, chronic stress responses may become maladaptive. Chronic stress leads to increased levels of glucocorticoids and prolonged sympathetic nervous system activation and has been associated with accumulation of visceral fat, type 2 diabetes, and related cardiovascular complications.

This chapter, dealing with activity of peripheral catecholaminergic systems under stress, is based on the materials presented in "Stress-Triggered Changes in Peripheral Catecholaminergic Systems" at the Tenth International Catecholamine Symposium held in Asilomar, Pacific Grove, California, USA, from 9 to 13 September 2012. The program was *chaired by Richard Kvetnansky* from the Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic, and had the following presentations:

- *Michael Ziegler* (University of California at San Diego, San Diego, USA): The Relationship of Norepinephrine to Stress-Induced Hypertension
- *Monika Ehrhart-Bornstein, Maria Rubin de Celis* (University of Dresden, Dresden, Germany): Chromaffin Progenitor Cells in the Adult Adrenal Medulla
- *Edmund La Gamma* (Children's Hospital at Westchester Medical Center, New York Medical College, New York, USA): Recurrent Hypoglycemic Stress Differentially Regulates Catecholamine Release and Transmitter Gene Expression
- *Magda Santana* (Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal): Chronic Unpredictable Stress Induces Catecholaminergic System Changes in Mouse Adrenal Gland
- *Tai T., Sandhya Khurana* (Northern Ontario School of Medicine, Laurentian University, Sudbury, Canada): Regulation of Adrenal Phenylethanolamine *N*-methyltransferase Gene Expression and Adrenaline Synthesis in a Fetal Programming Model of Hypertension
- *Steven N. Ebert* (University of Central Florida College of Medicine, Orlando, Florida, USA): Adrenergic Derived Myocardium: Anatomical Substrate for Stress-Induced Cardiomyopathies

New findings from these research groups are incorporated into the following review.

2. PERIPHERAL CATECHOLAMINERGIC SYSTEMS

In contrast to Cannon's original concept of a unitary sympathoadrenal system, which stated that the adrenal medulla and sympathetic nervous system functioned as a unit, there are several peripheral catecholaminergic systems that can be differently regulated by various stressors (Goldstein, 2003, 2010; Goldstein & Kopin, 2008; Kvetnansky, Sabban, & Palkovits, 2009). The peripheral sympathetic system consists of the following: (A)

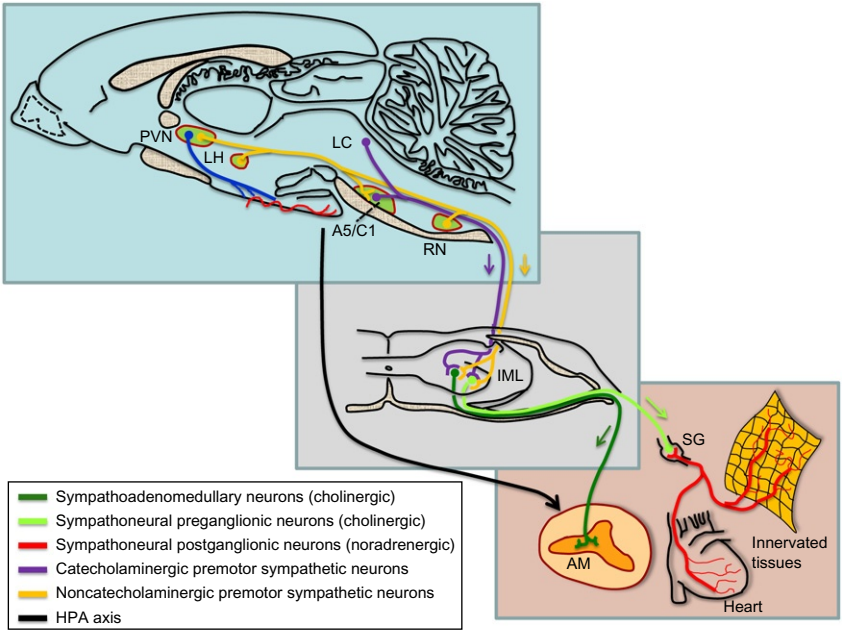

Figure 17.1 Nerve pathways that control peripheral sympathoadrenal activity. PVN, paraventricular nucleus of the hypothalamus; LC, locus coeruleus; IML, intermediolateral cells of the spinal cord; AM, adrenal medulla; SG, sympathetic ganglion.

sympathoadrenomedullary system, (B) sympathoneural system, (C) DOPA-dopamine autocrine/paracrine system, and (D) nonneuronal catecholaminergic systems (Goldstein, 1995; 2001; Kvetnansky et al., 2009; Fig. 17.1).

2.1. Sympathoadrenomedullary system

The adrenal medulla produces about 80% epinephrine (E) and 20% norepinephrine (NE) and is the major source of E in the circulation. The chromaffin cells in the adrenal medulla are innervated by the sympathetic preganglionic neurons from the intermediolateral cell column, mainly in thoracic segments T5–T9. The major central catecholaminergic input arises from the sympathetic premotor neurons (A5 noradrenergic and C1 adrenergic neurons; Fig. 17.1). The adrenal medulla responds to stressors like immobilization, hypoglycemia, emotional distress, shock, and fear.

The effects of various stressors on adrenomedullary catecholamine levels have been determined in early studies. Exposure to a single episode of

immobilization stress for up to 2 h is accompanied by a decrease of 15–20% in the total adrenal medullary content of E without significant changes in NE (Kvetnansky & Mikulaj, 1970). Following repeated exposures to the same stressor, the catecholamine levels in the adrenal medulla return to normal and even establish higher basal levels (Kvetnansky, Weise, & Kopin, 1970). The adrenal medulla responds to repeated immobilization stress with an increased capacity to synthesize catecholamines.

Stress alters the synthesis of catecholamines within the adrenal medulla through activity of three key catecholamine-synthesizing enzymes: tyrosine hydroxylase (TH), dopamine-beta-hydroxylase (DBH), and phenylethanolamine *N*-methyltransferase (PNMT) (Fig. 17.2). Activity of L-aromatic amino acid decarboxylase, in contrast, is unchanged with stress. The human adrenomedullary response to stress can be quite specific. Public

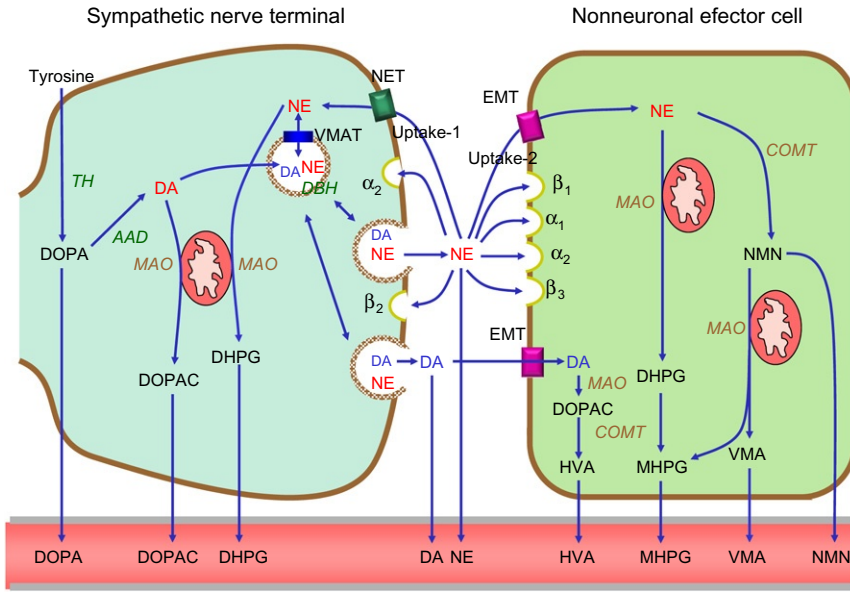

Figure 17.2 Biochemical pathways regulating synthesis, release, reuptake, and metabolism of catecholamines. AAD, amino acid decarboxylase; VMAT, vesicular monoamine transporter; DOPAC, dihydroxyphenylacetic acid; DHPG, dihydroxyphenylglycol; NET, norepinephrine transporter; COMT, catechol-*O*-methyltransferase; NMN, normetanephrine; MAO, monoamine oxidase; MHPG, 3-methoxy-4-hydroxyphenylglycol; HVA, homovanillic acid; VMA, vanillylmandelic acid.

speaking primarily increased plasma E, while a bout of exercise in the same subjects primarily increased plasma NE (Dimsdale & Moss, 1980).

Several reviews deal with various aspects of the regulation of catecholamine biosynthetic enzyme activity, protein levels, gene expression, and molecular genetics in response to stress (Goldstein, 2010; Kobayashi & Nagatsu, 2005; Kvetnansky & McCarty, 2007; Kvetnansky & Sabban, 1993, 1998; Kvetnansky et al., 2009; Sabban, 2007; Sabban, Hiremagalur, Nankova, & Kvetnansky, 1995; Sabban & Kvetnansky, 2001; Wong & Tank, 2007).

2.2. Sympathoneural system

The main neurotransmitter of the sympathoneural system is NE. Postganglionic neurons of the sympathoneural system innervate the majority of organs and produce and release NE (Fig. 17.1). The sympathoneural system is implicated in many of the pathophysiological responses to stress, such as hypertension and cardiac arrhythmias. This system has been less studied in stress conditions than the sympathoadrenomedullary system due to its diffuse anatomy. The sympathoneural system responds to stressors like cold exposure, exercise, hypotension, hemorrhage, pain, and hypovolemia.

Early studies established that exposure of rats to a variety of stressors triggers increased TH and DBH enzymatic activity in a number of sympathetic ganglia, including the superior cervical and stellate ganglia. Kiran and Ulus (1992) revealed selectivity in the response of TH activity in different sympathetic ganglia after exposure to various stressors. These stressors were also associated with elevated TH immunoreactive protein and elevated levels of TH and DBH mRNA levels (Kvetnansky et al., 2004; Nankova et al., 1996). Under conditions of repeated immobilization, increased TH protein and mRNA levels were observed in both stellate and superior cervical ganglia (Nankova et al., 1996). Thus, increased catecholamine biosynthetic capacity in the sympathetic nervous system likely helps mediate the stress-triggered elevation of NE in the plasma and in target tissues.

PNMT activity was also detected in the sympathetic ganglia of newborn (Paivarinta, Pickel, Eranko, & Joh, 1989) and adult rats (Culman, Torda, Petrikova, & Murgas, 1988; Schalling et al., 1991) and the activity increased after corticosterone administration. Nevertheless, convincing data concerning PNMT gene expression in stellate ganglia of adult rats and mice were published only recently (Kubovcakova et al., 2006; Kvetnansky et al., 2006). PNMT gene expression and PNMT protein levels in the

stellate ganglia increased after exposure to a single and especially after repeated immobilization stresses (Kubovcakova et al., 2006).

Severe acute stress reactions can precipitate a syndrome of heart failure and transient left ventricular systolic dysfunction (Wittstein, 2012). The syndrome of stress cardiomyopathy gives symptoms similar to an acute coronary syndrome but has unique clinical features that can readily be distinguished from acute infarction. Stress cardiomyopathy is reversible and occurs in the absence of plaque rupture and coronary thrombosis. Exaggerated sympathetic stimulation may play a pathogenic role in the development of stress cardiomyopathy. Plasma NE levels have been markedly elevated in patients with stress cardiomyopathy (Wittstein, 2012), and the syndrome has also been observed in other clinical states of catecholamine excess such as central neurological injury and pheochromocytoma. Much less severe stressors can nevertheless precipitate cardiac arrhythmias in subjects with heart disease.

2.3. Peripheral DOPA-dopamine autocrine/paracrine systems

Dopamine (DA) plays an important role as a neurotransmitter in the brain; however, understanding of DA functions in the periphery has lagged behind. DA is present in only quite small concentrations in the adrenal gland compared with E and NE. Plasma DA concentrations are, however, similar to those of E. Plasma DA is derived substantially from the sympathetic noradrenergic nerves via exocytosis; about 50–90% of plasma DA has a sympathoneural source (Goldstein & Holmes, 2008; Fig. 17.2). The vesicles undergoing exocytosis from the sympathetic nerves are estimated to contain about 25–50 times more NE than DA (Goldstein, 2010; Goldstein & Holmes, 2008). During orthostasis, individual plasma DA levels positively correlated with changes seen in NE levels. Stressors that elicited release of NE from the sympathetic nerves produced much larger increases in plasma NE levels than in plasma DA levels (Goldstein, 2010; Goldstein & Holmes, 2008).

Most DA production in the body occurs in nonneuronal cells, for example, in kidneys where DA appears to depend mainly on uptake of its precursor L-DOPA from the circulation with conversion to DA by the enzyme L-aromatic-amino acid decarboxylase in the proximal tubules—that is, nonneuronal and nonchromaffin cells (Wolfovitz et al., 1993). In humans, virtually all DA in urine comes from renal uptake and decarboxylation of L-DOPA. DA exiting the nonneuronal cells then appears to act as an

autocrine/paracrine substance, promoting natriuresis by local inhibition of Na^+K^+ATPase.

The presence of DA and its receptors was described also in the stomach, pancreas, and other mesenteric organs (Mezey et al., 1996, 1999) where DA contributes to regulation of gastrointestinal motility. The function of this system in stress situations is not clear.

2.4. Nonneuronal catecholaminergic systems

2.4.1 Nonneuronal catecholamine cell types

Patients with heart transplants maintain adequate cardiac function even in the absence of sympathetic reinnervation (Goncalvesova et al., 2004). In 1996, a new type of cardiac cell capable of adrenergic paracrine signaling was identified in mammalian hearts. These cells, which do not have neuronal or chromaffin cell ultrastructural morphology, were named ICA (intrinsic cardiac adrenergic) cells (Huang et al., 1996). They contain mRNA and proteins of enzymes involved in catecholamine biosynthesis including PNMT and may participate in cardiac regulation independently of sympathetic innervation. ICA cells are capable of catecholamine synthesis and uptake. E released from ICA cells helps regulate the rate of beating of cardiac myocytes. ICA cells occur in fetal myocardium in much higher abundance compared to adult heart (Saygili et al., 2011).

PNMT synthesizes E and is found in low amounts in many nonadrenal tissues such as the cardiac atria and ventricles, spleen, kidney, lung, thymus, skeletal muscles, skin, human red blood cells, lymphocytes, and fat cells and also in the sympathetic ganglia (Kubovcakova et al., 2006; Kvetnansky et al., 2006). Transient PNMT expression was also described in cardiomyocytes during embryogenesis, prior to establishment of the sinoatrial and atrioventricular nodes and sympathetic innervation (Fig. 17.3; Ziegler, Bao, Kennedy, Joyner, & Enns, 2002).

Recently, two types of catecholamine-containing intrinsic ganglionic neurons have been observed: the small intensely fluorescent (SIF) cells and large-diameter neurons (Slavikova, Kuncova, Reischig, & Dvorakova, 2003). SIF cells exhibit TH immunoreactivity, but they are not positive for DBH. In contrast, large-diameter intrinsic TH-positive neurons also display DBH-IR and PNMT-IR, thus indicating the capacity for the synthesis of NE and E. A majority of these large-diameter intrinsic neurons also show neuropeptide Y (NPY)-IR (Slavikova et al., 2003). SIF cells are most probably dopaminergic, whereas large-diameter intrinsic cells seem to represent a subpopulation of NE- or E-releasing neurons. Even if TH has been clearly

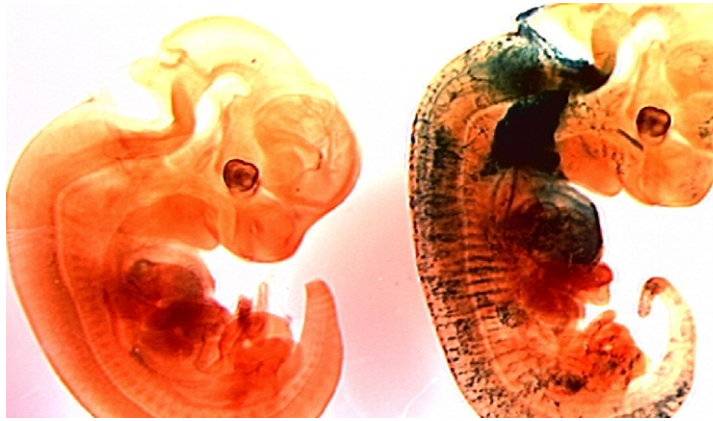

Figure 17.3 PNMT expression at embryonic day 12 in a mouse with the PNMT promoter linked to Cre recombinase to trigger the lacZ reporter compared with a control mouse. Note the prominent staining of chromaffin cells migrating from the neural crest. There is dense staining of the forming sympathetic chain. The heart chambers are outlined by dense staining that does not appear to originate in neural crest cells.

shown in ICA and SIF cells (Huang et al., 1996; Slavikova et al., 2003), TH gene expression was detected only in fetal rat hearts before sympathetic innervation and not in the heart of adult animals. However, PNMT was localized in adult cardiomyocytes (Fig. 17.4; Kennedy & Ziegler, 1991; Krizanova et al., 2001; Kvetnansky et al., 2004).

These findings have definitely confirmed an extra-adrenal presence of an E-producing system including the enzyme PNMT in the heart. The findings have also suggested that cardiac PNMT is an extraneuronal enzyme (Kennedy, Elayan, & Ziegler, 1993a; Krizanova et al., 2001; Kvetnansky et al., 2004; Tillinger et al., 2006; Ziegler, Kennedy, & Houts, 1998). PNMT was localized immunofluorescently in isolated cardiomyocytes and PNMT enzyme activity was present in adult cardiac atria (Kennedy & Ziegler, 1991; Tillinger et al., 2006). Extra-adrenal PNMT is present in many human tissues, where tissue enzyme levels correlate with E levels. The human PNMT is responsive to glucocorticoid stimulation and red blood cell levels of PNMT are increased in hyperthyroidism (Kennedy, Bigby, & Ziegler, 1995).

2.4.2 Embryogenesis

During embryogenesis, there is a burst of PNMT enzymatic activity in the heart of mice and rats peaking at day 12 (Fig. 17.3; Kennedy & Ziegler,

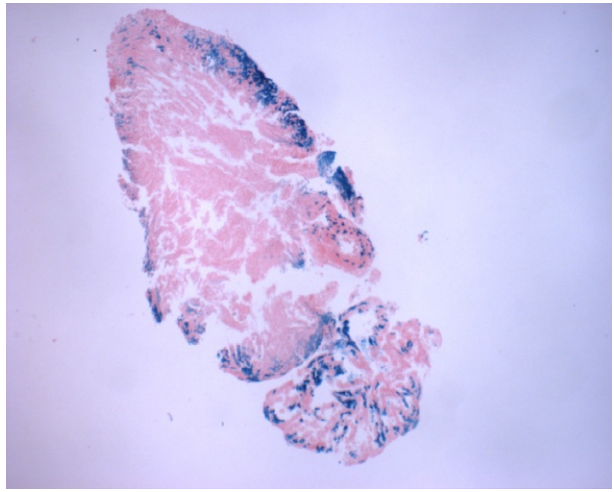

Figure 17.4 PNMT expression in the heart of an adult mouse with the PNMT promoter linked to Cre recombinase to trigger the lacZ reporter. Note the staining in the atria and along the conduction system.

1991). This can be further induced by administration of the glucocorticoid dexamethasone to the mother. This induction of PNMT activity can be seen in the adult as well. Glucocorticoids induce PNMT mRNA in the lung and PNMT enzymatic activity in the bronchial epithelial cells in culture. This induction of PNMT activity is seen in the arteries, skeletal muscle, and heart (Kennedy & Ziegler, 1991). The increase in PNMT enzymatic activity following glucocorticoid is closely paralleled by an increase in blood pressure (Kennedy, Elayan, & Ziegler, 1993b). The response of PNMT activity to glucocorticoids is due to the three glucocorticoid response elements in the promoter of PNMT. The question of whether E is responsible for the hypertension and insulin resistance seen after glucocorticoids is perhaps best addressed by study of PNMT knockout animals.

2.4.3 Epinephrine knockout models

The PNMT knockout mouse has undetectable E levels. When this animal is at rest, it has a normal blood pressure. During exercise, the PNMT knockout animal developed a greater increase in blood pressure than control animals (Bao et al., 2007) and remodeling of the left ventricle. This indicates that E protects against stress-induced hypertension, probably by stimulation of

vasodilating β_2-receptors. Not only did E protect against blood pressure increase during exercise, but also endogenous E protected against diabetes (Ziegler, Milic, Sun, et al., 2011). These experiments demonstrate that E may actually protect against the metabolic syndrome (Ziegler, Elayan, Milic, Sun, & Gharaibeh, 2012), in part by protecting against obesity-induced insulin resistance. Although the short-term effect of pharmacological doses of E is to increase blood glucose and diminish insulin sensitivity, long-term knockout of E production exacerbated diet-induced hyperglycemia and insulin resistance. There is reason to believe this is due to E stimulation of β_2-receptors. E stimulates β_2-receptors better than NE but has similar potency at α-, β_1-, and β_3- receptors. A chronic E infusion enhanced rat muscle insulin sensitivity. β_2-agonists in pharmacological doses lead to muscle hypertrophy in rats, cattle, pigs, poultry, and sheep by decreasing breakdown of muscle protein. E facilitates insulin binding to exercising muscle and increases muscle blood flow. The short-term effect of E is to raise blood pressure and blood sugar. The longer-term effect of E is the opposite. These long-term effects can be mimicked by the administration of β_2-agonists. Stimulation of β_2-receptors induces the glucose transporter GLUT4 and increases muscle mitochondria, permitting muscle to take up and metabolize glucose. In addition, β_2-receptors in the vasculature cause vasodilation, lowering blood pressure and increasing cardiac output. Thus, although it was once suspected that E might increase blood pressure and cause diabetes, over the long term, E appears to lower both blood pressure and blood sugar. This might account for some of the beneficial effect of exercise to prevent both hypertension and diabetes. Human athletes have used and abused the muscle growth-stimulating effects of β_2-agonist drugs, leading to regulations prohibiting their use in many professional sports and in the Olympics.

2.4.4 The effect of stress on nonneuronal catecholamine systems

The cardiac nonneuronal system producing E is also affected by stress. Stress exposure induced PNMT gene expression not only in the adrenal medulla but also in the cardiac atria and ventricles (Elayan, Kennedy, & Ziegler, 1990; Krizanova et al., 2001; Kvetnansky et al., 2006) and in the spleen (Jelokova et al., 2002). PNMT mRNA levels in heart tissues were several fold increased by immobilization stress (Krizanova et al., 2001; Kvetnansky et al., 2006). In the atria, PNMT mRNA levels were increased by hypoxia but decreased by cold stress. In the ventricles, no significant changes were observed. In the atria, gene expression of PNMT is clearly

modulated by glucocorticoids, because adrenalectomy or hypophysectomy prevents the increase in PNMT mRNA levels in response to immobilization stress. Levels of PNMT mRNA remain elevated in the cardiac atria of repeatedly immobilized rats and mice (Kvetnansky et al., 2006). This finding suggests that increased E synthesis in the heart of mammals is a part of the adaptation process of the organism to chronic or repeated stress exposure.

It is estimated that about one-third of cardiac E is synthesized by the heart itself. However, there are two different enzymes in the heart that can synthesize E. Atrial E-forming activity resembled adrenal PNMT. The ventricular tissue nonspecifically methylated both NE and DA. These results help explain why PNMT mRNA is found in the atria much more than in the ventricles, even though both synthesize E. A nonspecific methylating enzyme in the cardiac ventricles can synthesize E, but the activity of that enzyme for E formation is much lower than PNMT activity in the atria (Elayan et al., 1990). PNMT mRNA levels in human myocardium were significantly higher during the first three years following cardiac transplantation as compared to further periods after the transplantation (Goncalvesova et al., 2004). A decrease in the PNMT gene expression with years after transplantation could be a consequence of the reinnervation process. The PNMT gene is also expressed in the lymphoid tissues such as the spleen and thymus and in sympathetic ganglia. In these organs, PNMT mRNA levels were also found to be elevated by stress (Kubovcakova et al., 2006; Kvetnansky et al., 2006).

Epinephrine production by extra-adrenomedullary tissues increased following stress. The primary mechanism appears to be corticosteroid induction of PNMT (Kvetnansky et al., 2006), which can act on NE released in response to stress. It is not clear how large a role this extra-adrenal E plays in relation to circulating E derived from the adrenal medulla. There is fair evidence for participation of cardiac E in pacemaking during embryogenesis, but PNMT activity in the heart is very high at day 12 of rat embryogenesis and declines thereafter.

2.4.5 Catecholamine systems in the adipose tissue

Catecholamines regulate lipolysis in the adipose tissue and are produced mainly by the sympathoadrenal system. Endogenous catecholamine production has also been found in nonneuronal cells of adipose tissues—in adipocytes (Vargovic et al., 2011). Rat adipocytes from mesenteric adipose tissue express genes of catecholamine biosynthetic enzymes and produce catecholamines *de novo (*Kvetnansky et al., 2012). Acute or chronic cold exposure

increased intracellular NE and E levels in isolated rat mesenteric adipocytes. A single immobilization stress caused increased NE, E, catecholamine biosynthetic enzymes, and vesicular monoamine transporter 1 (VMAT1), but repeated exposure to immobilization stress showed a highly exaggerated effect (Vargovic et al., 2013). In the adipose tissues, both the sympathoadrenal system and adipocyte catecholamine systems can produce catecholamines, and both systems are activated by stress.

2.5. Adaptation to specific stressors

In 1936, Selye described a pathological triad—adrenal enlargement, gastrointestinal ulceration, and thymicolymphatic involution—which should be elicited by any stressor. In 1989, Vigas proposed the idea that stress responses are specific, which modified Selye's theory of stress. More than a half century elapsed before Selye's doctrine of nonspecificity underwent testing by an NIH group, which failed to confirm it (Pacak et al., 1998). Stressor-specific responses of catecholamine systems have been described (Kvetnansky, Pacak, Sabban, Kopin & Goldstein, 1998; Pacak & Palkovits, 2001; Pacak et al., 1998). Goldstein recently introduced a new definition of stress (Goldstein, 2003; Goldstein & Kopin, 2007). Central to his stress theory is that the body possesses numerous homeostatic comparators, which have been called "homeostats." Different homeostats can regulate the activity of the same effector system. The definition of stress formulated by Goldstein is: "Stress is a condition in which expectations, whether genetically programmed, established by prior learning, or deduced from circumstances, do not match the current or anticipated perceptions of the internal or external environment, and this discrepancy between what is observed or sensed and what is expected or programmed elicits patterned, compensatory responses." Allostasis is the process of adaptation of the body upon the exposure to various stressors (Goldstein, 2003; Goldstein & Kopin, 2007; McEwen 1998; 2004).

In general, stressors can be divided into four main categories (Pacak & Palkovits, 2001):

1) *Physical stressors*, for example, cold, heat, radiation, noise, vibration, chemical stressors, pain, and immobilization.
2) *Psychological stressors* that affect emotional processes and may result in behavioral changes such as anxiety, fear, or frustration. This can be triggered in animals by handling or restraint.

3) *Social stressors*, reflecting disturbed interactions among individuals, for example, unemployment, marital separation, death of partner, and dominancy in animals.
4) *Stressors that challenge cardiovascular and metabolic homeostasis*, for example, exercise, orthostasis, upright tilt, hypoglycemia, and hemorrhage.

Different stressors elicit different patterns of activation of the sympathetic nervous, adrenomedullary, hypothalamic–pituitary–adrenocortical (HPA), and other effectors.

In terms of duration, stressors may be either

(a) *acute stressors* (single, intermittent, and time-limited exposure) or
(b) *chronic or repeated stressors* (continuous long-term prolonged exposure and intermittent long-term exposure).

There are different specific responses of sympathoadrenomedullary, HPA, and sympathoneural systems to various stressors from different categories. This specificity in responses of the organism to various stressors exists not only at the level of plasma E, NE, and corticosteroids (Kvetnansky, 2004; Kvetnansky et al., 1998; Pacak & Palkovits, 2001; Pacak et al., 1998) but also at the level of gene expression and transcription factors of enzymes involved in catecholamine biosynthesis (Kvetnansky, 2004; Kvetnansky, Jelokova, et al., 2002; Kvetnansky, Nankova, et al., 2002; Liu, Kvetnansky, Serova, Sollas, & Sabban, 2005; Sabban, Hebert, Liu, Nankova, & Serova, 2004; Sabban, Nankova, Serova, Kvetnansky, and Liu, 2004; Wong & Tank, 2007).

Goldstein and Kopin (2008) reported results of a meta-analysis of the literature examining interrelationships among responses to stressors, as measured by plasma E, adrenocorticotropic hormone (ACTH), and NE levels. Mean E responses were strongly positively correlated with mean ACTH responses ($r=0.93$) and less strongly with NE responses ($r=0.40$). Plasma E responses were disproportionately larger than NE responses during hypoglycemia and smaller than NE responses during cold exposure without hypothermia, orthostasis, and active escape/avoidance. Plasma NE responses were disproportionately larger than ACTH responses during cold exposure without hypothermia and severe/exhausting exercise and smaller than ACTH responses during hypoglycemia. The results of this meta-analysis indicate a close association between adrenomedullary and HPA responses across a variety of stressors. This association seems to be stronger than that between adrenomedullary and sympathetic noradrenergic responses. The findings therefore favor the concept of a unitary adrenal system over that of a unitary sympathoadrenal system (Goldstein & Kopin, 2008).

3. THE EFFECT OF STRESS ON ORGAN SYSTEMS

3.1. Release of catecholamines in stress

Individual stressors such as heat, cold, hypoglycemia, exercise, hemorrhage, and psychological challenges require specific responses. Appropriately, different stressors elicit different patterns of activation, closing negative feedback loops. Regulation of E and NE release is highly stressor specific. Hypoglycemia and immobilization elicited mainly E release. However, stressors such as cold and pain induced NE release (Goldstein & Kopin, 2008). Medullary cellular hypertrophy, but not hyperplasia, is a general consequence of chronic stress.

The mechanism of release of catecholamines is similar in the adrenal medulla and the sympathetic nerve endings. Acetylcholine released from the sympathetic preganglionic nerve terminals binds to nicotinic cholinergic receptors and leads to a depolarization of the cell membrane, resulting in an increase in membrane permeability to sodium. This initiates a series of events that lead to an increase in the influx of calcium. Then, catecholamine storage vesicles fuse with the chromaffin or sympathetic neuronal cell membranes and, via exocytosis, release their contents of catecholamines, together with chromogranins, other neuropeptides, ATP, and a fraction of the soluble DBH. NE and ATP are stored in both small synaptic and large dense-core vesicles, but neuropeptides are stored only in the large ones. Release of NPY, therefore, does not parallel that of NE and ATP, as exocytosis from small and large vesicles is regulated differently (Zukowska-Grojec, 1995). After exocytosis, the vesicle membrane is retrieved from the plasma membrane and recycled into newly formed vesicles.

Following exocytosis, catecholamines that escape reuptake and local metabolism diffuse into the circulation and constitute the circulating pool of catecholamines. Plasma catecholamines turn over very rapidly. The half-time of disappearance of human NE is about 2.5 min (Esler et al., 1979). Under resting conditions, low levels of catecholamines are released into the blood from the adrenal medulla and sympathetic nerve terminals. During stressful stimulation, however, a huge amount of E (about 95%) and a significant amount of NE (which may comprise up to 30% of the total circulating NE) may be released from the adrenal medulla. The remaining 70% of NE is released from the sympathetic nerve terminals and enters blood capillaries from the site of release at the neuroeffector junction. Human plasma normally contains six catecholamines: E, NE, and DA; the catecholamine precursor

L-DOPA; the metabolite of NE—dihydroxyphenylglycol (DHPG); and a metabolite of DA—dihydroxyphenylacetic acid (DOPAC) (Fig. 17.2).

Plasma NE is released mainly from the sympathetic nerves and the majority of NE is metabolized before entry into plasma. Plasma NE levels depend both on the rate of NE release into plasma and the rate of its removal from the plasma. In some pathological situations, NE may be released from the sympathetic terminals by a nonexocytotic mechanism.

Plasma E levels parallel neural outflow to the adrenal medulla. Under the effect of various stressors, E responses are more closely related to ACTH levels than to NE. The fate of E that enters the bloodstream differs quantitatively from that of NE. E is a poorer substrate than NE for uptake-1 and a better substrate than NE for extraneuronal uptake-2. E is also a better substrate than NE for catechol-*O*-methyltransferase (COMT). Therefore, more of circulating E than NE is metabolized by extraneuronal uptake and *O*-methylation (Goldstein, 2010).

Plasma DA levels are low and similar to those of E, but circulating DA does not act as a hormone (Goldstein, 2010). Stressors that elicit release of NE from sympathetic neurons produce much larger increases in plasma NE levels than in plasma DA levels. Free plasma DA is mostly released from the sympathetic nerves (Goldstein & Holmes, 2008). In humans, at least 95% of circulating DA is present in sulfoconjugated form (Goldstein, 2010). Levels of DA sulfate respond relatively little to acute exposure to various stressors, for example, exercise. However, meal ingestion markedly increases plasma DA sulfate levels (Goldstein, 2010).

Plasma DOPA levels exceed those of NE by about tenfold, due to more rapid clearance of NE than of L-DOPA from plasma. L-DOPA is the precursor of catecholamines and the product of the rate-limiting step in biosynthesis. Immobilization stress in rats increases L-DOPA levels in plasma within a few minutes, and blockade of catecholamine biosynthesis prevents these increases (Kvetnansky, Armando, et al., 1992).

Plasma DHPG is formed from NE in sympathoneural cytosol by deamination and reduction. DHPG diffuses rapidly across cell membranes into the extracellular fluid and then to the bloodstream. Plasma DHPG levels offer a biochemical index of NE turnover (Goldstein, 2010).

Plasma DOPAC is a DA metabolite. DOPAC levels are about 50 times higher than DA, due to much slower clearance of DOPAC than of DA from the circulation. Immobilization stress in rats rapidly increases plasma DOPAC levels (Kvetnansky, Goldstein, et al., 1992), and blockade of catecholamine biosynthesis prevents the stress-induced increases in plasma

DOPAC (Kvetnansky, Armando, et al., 1992). Plasma DOPAC might also be formed from metabolism of DA in nonneuronal cells of the gastrointestinal tract.

Decapitation of animals produced an 80-fold increase in plasma E and an eightfold increase in plasma NE levels compared to values obtained from blood collected via a permanently inserted arterial catheter (Kvetnansky et al., 1978). Even minor disturbances like handling or transfer of animals produce highly significant increases in E and NE levels (Kvetnansky et al., 1978). Many stressors increase not only plasma catecholamine levels but also the catecholamine precursor DOPA and catecholamine metabolites. This indicates that stress increased catecholamine synthesis, release, and metabolism. Adrenal medullectomy completely prevents the stress-induced increases in plasma E but reduces plasma NE levels only by about 30%. Combined adrenal medullectomy and sympathectomy almost completely abolishes plasma NE. Therefore, during stress, the increment in plasma E is derived almost completely from the adrenal medulla, whereas most plasma NE (about 70%) is derived from the sympathetic nerves (Kvetnansky, Weise, Thoa, & Kopin, 1979). Regulation of E and NE release is highly stressor-specific (Goldstein & Kopin, 2008). Adrenal medullary E release is mainly induced by hypoglycemia, immobilization, and emotional stressors (Kvetnansky et al., 1998; Pacak et al., 1998). Conversely, cold or pain exposure does not activate E release but highly stimulates NE release (Kvetnansky et al., 1998; Pacak et al., 1998).

When immobilization stress is applied daily for several weeks, baseline levels of plasma catecholamines are elevated but the stress-induced increment is reduced (Kvetnansky, Nemeth, Vigas, Oprsalova, & Jurcovicova, 1984; Stone & McCarty, 1983). Repeated handling diminished E and ACTH responses but not NE responses compared to the first handling procedure (Dobrakovova, Kvetnansky, Oprsalova, & Jezova, 1993). When handling-adapted animals are handled by a different person, E responses are enhanced. This dissociation of plasma E and NE responses illustrates differing control of sympathoadrenomedullary and sympathoneural systems. Rats exposed to repeated immobilization have significantly increased plasma DBH activities (Kvetnansky et al., 1984; Weinshilboum, Kvetnansky, Axelrod, & Kopin, 1971). An exaggerated response of plasma catecholamines occurred in rats adapted to a homotypic stressor after a heterotypic novel stressor (Dronjak, Ondriska, Svetlovska, Jezova, & Kvetnansky, 2002). Thus, a novel stress can exaggerate the response to an ongoing stress.

3.2. Inactivation and uptake of catecholamines in stress

The physiological effects of catecholamines released into the synaptic cleft are terminated very rapidly by uptake back into the sympathetic nerve endings and effector cells with some conversion to inactive metabolites. Sympathetic nerve endings take up catecholamines from the extracellular fluid by a process distinct from the intraneuronal uptake of catecholamine by the storage granules. Neuronal uptake is known as "uptake-1" (Eisenhofer, 2001) and the uptake by nonneuronal tissues as "uptake-2" (Iversen, 1965).

Uptake-1 serves to recapture NE (Fig. 17.2). The carrier can transport NE against large concentration gradients. This uptake plays a less important role in the inactivation of circulating E. Uptake-1 increases in parallel with increased NE release during exposure of the organism to stressors (Eisenhofer, Cox and Esler, 1990; Eisenhofer, Esler, et al., 1991; Eisenhofer, Kopin, Goldstein, 2004b). About 90% of released NE is taken up by neurons (Eisenhofer et al., 1990; Eisenhofer, Esler, et al., 1991; Eisenhofer, Goldstein, Kopin, 1989; Eisenhofer, Smolich, Cox, Esler, 1991) mediated by NE transporter (NET) and DA transporter (DAT) proteins. NE is translocated by NET about twofold more effectively than E. DA is a much better substrate for DAT than NE or E.

Extraneuronal uptake-2 (U-2) is an active process of transport into nonneuronal cells. The extraneuronal monoamine transporter of uptake-2 (Iversen, 1965) has little if any stereospecificity and has low affinity (higher K_m). Uptake-2 favors E over NE and is not a Na^+- or Cl^--dependent process. Uptake-2 is sensitive to inhibition by *O*-methylated metabolites normetanephrine and metanephrine and by corticosteroids. Uptake-2 is responsible for formation of catecholamine metabolites in the liver, kidney, and lung and is highly sensitive to inhibition by glucocorticoids. It now appears that there are at least three nonneuronal catecholamine transporters functioning at extraneuronal locations. The classic transporter is corticosterone sensitive—the uptake-2 transporter, also referred as OCT3 "organic cation transporter." Other two extraneuronal organic cation transporters, which also transport catecholamines, were identified as OCT1 and OCT2.

Vesicular monoamine transporters (VMAT). Varicosities in the peripheral sympathetic neurons contain cytoplasmic vesicles. These vesicles actively store synthesized or recaptured cytoplasmic catecholamines and have specific carrier proteins called vesicular monoamine transporters (Johnson, 1988). Cloning studies revealed the existence of two isoforms of this transporter—VMAT1 (the "neuroendocrine" isoform) and VMAT2 (the "neuronal" isoform) (Eiden et al., 2002; Schuldiner, 1994). These two VMAT isoforms are encoded

by separate genes with different cellular distribution. Neurons express only VMAT2. In contrast, the adrenal medullary chromaffin cells express both isoforms with VMAT1 predominant in rodents and VMAT2 in humans. Paracrine SIF cells of the sympathetic ganglia express predominantly the VMAT1 isoform (Eiden et al., 2002). Catecholamines are, in general, better substrates for VMAT2 than for VMAT1 (Goldstein, 2001). The energy for vesicular uptake by VMAT is provided by the proton gradient, and the acidic vesicles attract basic amines. This is in contrast to transport of neurotransmitters across the plasma membrane (NET), which is sodium dependent. Under basal conditions, VMAT1 is widely expressed in all adrenal chromaffin cells while VMAT2 is colocalized with TH but not PNMT, indicating its expression in NE, but not E-synthesizing chromaffin cells. After exposure to immobilization stress, VMAT2 mRNA together with TH mRNA was elevated, reflected by increased VMAT2. The stress-induced expression of the VMAT2 gene could be mediated by a rise in glucocorticoids (Sabban, Tillinger, Nostramo, & Serova, 2012; Tillinger, Sollas, Serova, Kvetnansky, & Sabban, 2010). Catecholamines taken up by monoamine cell membrane transporters are then transported into storage vesicles or metabolized by monoamine oxidase (MAO) in the cytoplasm of neurons or by COMT in nonneuronal cells (Fig. 17.2). Eisenhofer, Kopin, and Goldstein (2004a) and Eisenhofer et al. (2004b) found increased NE release and reuptake but unchanged NE leakage from storage vesicles during exercise, indicating increased use of NET and VMAT during stress (Eisenhofer et al., 2004a).

Under basal conditions, VMAT1 is widely expressed in all adrenal chromaffin cells while VMAT2 is colocalized with TH but not PNMT, indicating its expression in NE, but not E-synthesizing chromaffin cells. After exposure to stress, there was no change in levels of VMAT1 mRNA. However, VMAT2 mRNA together with TH mRNA was elevated after exposure of rats to a single or repeated immobilization for 6 days. The changes in VMAT2 mRNA were reflected by increased VMAT2 protein (Sabban et al., 2012; Tillinger et al., 2010). Sabban et al. (2012) have shown that the stress-induced expression of the VMAT2 gene could be mediated by a rise in glucocorticoids.

3.3. Degradation and vesicular leakage of catecholamines in stress

Catecholamines are subjected to chemical degradation by 3-*O*-methylation (COMT, EC 2.1.1.6) and oxidative deamination (MAO, EC 1.4.3.4) and

by conjugation as sulfate and glucuronide. After neuronal reuptake, cytoplasmic NE can undergo metabolism catalyzed by MAO to form DHPG or translocation back into the storage vesicles via VMAT. The latter constitutes the predominant pathway (see Fig. 17.2) where MAO catalyzes deamination of amines, with production of aldehydes that are metabolized to carboxylic acids or alcohols. The MAO-A subtype has a higher affinity for NE and E. MAO-B is responsible for degradation of DA; both are mainly localized in the liver. MAO participates in regulation of NE storage in the nerve terminals. COMT is primarily an extraneuronal enzyme, which metabolizes circulating catechols, mainly in the liver and kidney. Phenolic hydroxyl groups of catecholamines can also be conjugated to sulfates or glucuronides. Eisenhofer and coworkers (2004a, 2004b) demonstrated that most metabolism of catecholamines takes place within the same cells in which the amines are synthesized. In *sympathetic nerves*, the aldehyde produced from NE by MAO is converted to 3,4-DHPG and not to 3,4-dihydroxymandelic acid. Subsequent extraneuronal *O*-methylation leads to production of 3-methoxy-4-hydroxyphenylglycol and not to vanillylmandelic acid (see Fig. 17.2). This acid is instead formed *in the liver* by oxidation catalyzed by alcohol and aldehyde dehydrogenases. Compared to intraneuronal deamination, extraneuronal *O*-methylation of NE and E represents a minor pathway of catecholamine metabolism.

Most of the MAO metabolite DHPG, produced under *resting conditions*, comes from NE leakage from vesicles. In the resting human heart, about 73% of NE turnover is due to intraneuronal metabolism of NE leaking from storage vesicles (Eisenhofer et al., 1998, 2004a; Eisenhofer & Lenders, 1998). Under *conditions of exercise* (at 50% of maximal work capacity), rates of NE release and reuptake exceed the rate of NE leakage from storage vesicles, which as a passive process operates independently of exocytotic release and remains relatively constant. Vesicular leakage may therefore be viewed as a mechanism that metabolizes catecholamines that are produced in preparation for stress responses. Because the ability to increase TH activity is limited, this leakage mechanism provides sympathetic nerves with a capacity for a more extended range of sustainable release rates than would otherwise be possible (Eisenhofer et al., 2004a; 2004b).

Immobilization stress decreased both MAO-A and MAO-B activities in rats (Obata & Yamanaka, 1994). Exposure to cold decreased liver MAO-A activity and also the ratio MAO-A/MAO-B. Decreased MAO-A activity and unchanged MAO-B activity are also observed in the hearts and brains of rats exposed to foot shock (Lemoine, Armando,

Brun, Segura, & Barontini, 1990). MAO activity was unchanged in the adrenal medulla of stressed animals (Kvetnansky, Torda, Jahnova, & Saleh, 1975). Animals immobilized repeatedly have shown lower COMT activity in both parts of the adrenal. MAO and COMT activities were also studied in the adrenals of rats that spent 18–20 days in space onboard three COSMOS biosatellites. The data suggest that a prolonged stay in weightlessness does not appear to be a stressful stimulus for catecholaminergic systems (Kvetnansky et al., 1981). MAO activity declined in the sympathetic ganglia of stress-predisposed animals after a stressor, while in stress-resistant animals, it increased (Gorbunova & Kashtanov, 1983). Thus, stress-induced changes in catecholamine-degrading enzyme activity are not uniform and depend on the strain of the animals, model of stress, timing of stressor, and many other factors. COMT activity (Kvetnansky et al., 1976) and also COMT mRNA levels were significantly decreased in the liver of adult mice exposed to stress (Mikhailova, Gulyaeva, & Filipenko, 2005). In aged mice, however, the effect of stress on COMT mRNA levels in the liver is absent (Mikhailova et al., 2005). Genetic differences in COMT may underlie individual differences in response to psychological and physical stressful challenges (Smolka et al., 2005; Zubieta et al., 2003). An association between COMT genotype and history of violent behavior has been seen in some human studies (Jabbi et al., 2007; Lachman, Nolan, Mohr, Saito, & Volavka, 1998), and COMT is a possible candidate gene involved in the pathogenesis of major depressive disorders and schizophrenia.

After exposure to stress, the activity of MAO and COMT is reduced in some organs. The reduced degradation process, together with stress-induced increases in catecholamine production, release, and secretion, might be involved in the enhanced availability of catecholamines for adrenergic receptors and for increased activity of metabolic and physiological processes under stress. Although the general trend is for catecholamine degradative enzymes to decrease with stress, the opposite is true for VMAT. This can lead to greater availability of NE and E and greater vesicular stores after stress. However, the cardiovascular consequences of inhibition of VMAT and MAO are not so straightforward. Inhibition of both can cause postural hypotension, even though their inhibition should increase intrasynaptic NE, leading to a pressor, not a depressor, effect. The postural hypotension could be due to intrasynaptic NE stimulation of presynaptic α_2 receptors, leading to inhibition of NE release, or due to the prominent central nervous effects of these drugs.

3.4. Renin activation and accumulated cell proliferation in the adrenal gland

All components of the renin–angiotensin system, including prorenin, renin, angiotensinogen, angiotensin-converting enzyme, and angiotensins I and II, were expressed in both the adrenal cortex and the adrenal medulla (Armando, Jezova, Bregonzio, Baiardi, & Saavedra, 2004). Local angiotensin II production could regulate the production of aldosterone and glucocorticoids and stimulate catecholamine secretion in the adrenal medulla (Armando et al., 2004). Angiotensin II served as a paracrine amplifier of the morphogenic signal and had a critical role in adrenocortical cellular proliferation and development.

3.5. Corticosteroid responses to stressors

Adrenocortical cells of adult mammals have low proliferative activity. Stress induces corticotropin-releasing hormone (CRH) release from the paraventricular nucleus of the hypothalamus, ACTH release from the anterior lobe of the pituitary gland, and glucocorticoid release from the adrenal cortex. Acute ACTH administration induced hyperplasia in the zona fasciculate of the adrenal cortex. Chronic stress affected not only ACTH but also the adrenal cortex. When animals were exposed to a continuous or intense stressor, ACTH gradually returned to normal levels. However, glucocorticoid levels remained high. Moreover, many patients with depression had increased basal plasma cortisol and enlarged adrenals.

3.6. The effect of maternal stress on offspring

The adrenal medulla develops internal to the adrenal cortex because of adrenocortical release of glucocorticoids. Transgenic animals that fail to express adrenomedullary glucocorticoids have dispersed chromaffin cells that fail to form a discrete adrenal medulla. Furthermore, the expression of PNMT and E synthesis depends on stimulation of glucocorticoid response elements in the PNMT promoter. Maternal stress increases glucocorticoid release through the HPA axis. The effect of maternal glucocorticoid excess is easily modeled by the administration of exogenous glucocorticoids. Glucocorticoids are used in obstetric practice, primarily to enhance lung maturation in cases of threatened preterm labor to reduce mortality in preterm infants. Although these treatments improve survival, they are not without adverse effects.

Prenatal exposure to glucocorticoids during their final week of pregnancy is sufficient to produce permanent adult hypertension in the rat (Levitt, Lindsay, Holmes, & Seckl, 1996). Even physiological levels of administered glucocorticoid for just 2 days were sufficient to develop hypertension in the rat offspring (Singh et al., 2007). The 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) enzyme, which serves as the placental barrier for glucocorticoid transfer, is present in syncytiotrophoblasts in the placenta of humans and many other mammalian species. It catalyzes the conversion of active glucocorticoids into inactive 11-keto metabolites, thereby protecting the fetus from elevated concentrations of maternal glucocorticoids. Nevertheless, increased levels of endogenous glucocorticoids can result in hypertension in the rat offspring (Singh et al., 2007).

Glucocorticoids regulate PNMT in two ways, posttranslationally by indirectly preventing the degradation of the PNMT enzyme and transcriptionally by regulating the expression of PNMT mRNA (Wong, Lesage, Siddall, & Funder, 1992). Dexamethasone induces PNMT mRNA as much as 20-fold. Though animal studies suggest that fetal exposure to glucocorticoids contributes to adult hypertension, long-term effects of fetal exposure to glucocorticoids in human are difficult to study but suggest a link to higher blood pressure after age 30.

3.7. Human models of chronic sympathetic nervous system stress responses

Patients with Alzheimer's can be disruptive and need careful supervision day and night. Spousal caregivers to Alzheimer's patients are placed in a stressful situation and frequently complain of work overload without respite. These caregivers have an accelerated risk of developing hypertension over a period of 5 years (Shaw et al., 1999). This may be a response to an increase in their allostatic load or in response to their sleep disturbance. Multivariate analysis shows a close relation between NE levels and sleep disturbance in caregivers. The severity of the increase in sympathetic nerve activity among caregivers is attested to by a decrease in sensitivity of their β_2-adrenergic receptor. The increased sympathetic nerve activity is accompanied not only by increased blood pressure but also by impaired function of their vascular endothelium and atherosclerosis (von Känel et al., 2011).

Sleep is disturbed in Alzheimer caregivers (von Känel et al., 2010) but is much more disturbed in obstructive sleep apnea. Patients with sleep apnea fail to lower their blood pressure at night as much as normal subjects and have increased blood pressure day and night (Ziegler, Milic, Sun, et al.,

2011). They have increased plasma NE throughout the day and night and desensitized β_2-adrenergic receptors. These sympathetic nervous system disorders are largely ameliorated by treatment of the sleep apnea with continuous positive airway pressure, which also lowers blood pressure. It is noteworthy that pharmacological treatment of hypertension that accompanies sleep apnea with drugs that block excess sympathetic nerve activity tends to be more effective than with other types of drugs (Ziegler, Milic, & Elayan, 2011). Slow-wave (deep) sleep is the period of least sympathetic nerve electrical activity, lowest heart rate, and lowest blood pressure. A study of elderly normotensive men found that those with the least deep sleep were the most likely to develop hypertension (Fung et al., 2011).

Psychological stress has long been considered a potential cause of human hypertension. Human studies suggest that severe stresses such as caregiving for Alzheimer's patients or sleep apnea might lead to hypertension through chronic activation of the sympathetic nervous system. Sleep disruption may play a role in the sympathetic nervous activation, since deep sleep is the period of least sympathetic nerve activity.

3.8. Hypoglycemia-associated autonomic failure

Medication-induced hypoglycemia affects most people with type 1 diabetes and many people with type 2 diabetes. Recurrent hypoglycemic stress can cause hypoglycemia-associated autonomic failure (HAAF), leading to both defective glucose counterregulation by reducing adrenomedullary E response and unawareness by reducing the symptomatic response to hypoglycemia. Three independent studies demonstrated that scrupulous avoidance of hypoglycemia reverses hypoglycemia unawareness and improves the reduced E component of defective glucose counterregulation in most affected patients. Recently, studies (Ramanathan & Cryer, 2011) demonstrated that adrenergic blockade prevented the effect of hypoglycemia to reduce the plasma catecholamine responses to subsequent hypoglycemia.

Recurrent hypoglycemia and the attendant defective counterregulation are the main limiting factors for long-term health benefits of intensive insulin therapy in diabetes. Recent studies from the Nankova lab by Edmund La Gamma compared the effects of single daily versus twice-daily episodes of insulin-induced recurrent hypoglycemia for 3 days in normal rats on adrenal E release and its production as reflected by altered TH mRNA levels, PNMT, and preproenkephalin (ppEnk, a costored and coreleased neuropeptide). Single daily episodes of recurrent hypoglycemia resulted in an

early-onset increase in plasma E, corticosterone, and glucagon with a corresponding later-onset upregulation of TH, PNMT, and ppEnk mRNA during a hypoglycemic clamp study on day 4. Exposure to twice-daily recurrent hypoglycemia reduced the immediate counterregulatory response of E and glucagon but not corticosterone. Attenuation was associated with a limited elevation of TH mRNA without affecting PNMT or ppEnk mRNA. The frequency of the antecedent hypoglycemic episodes can either increase TH mRNA (once daily), to sustain biosynthesis and thus the releasable pool of E or, paradoxically, attenuate its elevation (twice daily). These mechanisms appear to require circulating factors *in vivo* not present in *in vitro* models. Nuclear run-on assays show butyrate-dependent decreases of both TH mRNA and TH protein in PC12 cells, the cultured adrenal cells from rat origin. This signaling may represent a significant contributing factor to the clinical syndrome of HAAF.

4. MOLECULAR GENETIC MECHANISMS OF PERIPHERAL CATECHOLAMINERGIC RESPONSES TO STRESS

4.1. Stress and induction of catecholamine biosynthetic enzymes

Increased expression of catecholamine biosynthetic enzymes is a prevalent response to many types of stressors. The adrenal medulla rapidly responds to single stressors by targeting individual genes in response to cholinergic stimulation. In contrast, the sympathetic ganglia are especially responsive to activation of the HPA axis (Sabban & Kvetnansky, 2001). Several reviews deal with various aspects of the regulation of CA biosynthetic enzyme activity, protein levels, gene expression, and molecular genetics during exposure to stressors (Kobayashi & Nagatsu, 2005; Kvetnansky & McCarty, 2007; Kvetnansky & Sabban, 1993, 1998; Kvetnansky et al., 2009; Sabban, 2007; Sabban et al., 1995; Sabban & Kvetnansky, 2001; Wong & Tank, 2007). The paraventricular, periventricular, and dorsomedial hypothalamic nuclei are important centers regulating neuroendocrine and autonomic systems during stress. Immobilization stress increased TH mRNA and TH protein levels in these brain nuclei and in the peripheral sympathetic nervous system (Kiss, Mravec, Palkovits, & Kvetnanský, 2008).

Administration of the transcriptional inhibitor actinomycin D to rats blocks the stress-evoked increases in adrenal DBH and TH activity and TH and PNMT mRNA levels. Repeated exposure to immobilization stress leads to increased enzymatic activity and TH and DBH protein levels. No

significant changes were observed with a single exposure to stress (Sabban & Kvetnansky, 2001). However, both single and repeated immobilization stress led to increased initiation of TH and DBH gene transcription to levels about threefold higher than in control animals. A single 5 min stressor caused a three- to fourfold increase in TH and DBH transcription rate. Thus, transcription of TH and DBH genes is almost as fast as the stress-triggered rise in levels of adrenal glucocorticoids. However, this rise is transient if the stress is not prolonged. Following repeated stress (2 h daily for several consecutive days), the activation of TH and DBH transcription is stabilized and significantly elevated even 24 h after the last stress exposure (Nankova & Sabban, 1999; Nankova, Tank, & Sabban, 1999). Repeated immobilization kept levels of TH and DBH mRNA elevated in both the sympathetic ganglia and the adrenal medulla, but did not produce further increase compared to already elevated levels in adapted control groups. Thus, elevated transcription of catecholamine biosynthetic enzymes plays an important role in response of the peripheral catecholaminergic systems to stressors.

Quantitative evaluation of gene expression of catecholamine biosynthetic enzymes (Kvetnansky et al., 2004) showed that *in the adrenal medulla*, the basal concentration of TH mRNA was about 0.5 amol/ng of total RNA. DBH mRNA was about 12 times higher (6.0 amol/ng RNA) and PNMT mRNA about 56 times higher (28.0 amol/ng RNA) than TH mRNA. *In the stellate ganglia*, the basal concentration of TH mRNA (0.02 amol/ng RNA) was about 25 times lower than in the adrenal medulla, but DBH mRNA in ganglia (2.6 amol/ng RNA) was present at similar concentration as in the adrenal medulla (Kvetnansky et al., 2004; Micutkova, Rychkova, Sabban, Krizanova, & Kvetnansky, 2003). Repeated immobilization (2 h daily for 7 days) kept levels of TH and DBH mRNA elevated both in the sympathetic ganglia and the adrenal medulla. PNMT gene expression was also increased after repeated stress in both the stellate ganglia and the adrenal medulla of rats and mice (Kubovcakova et al., 2006; Kvetnansky et al., 2006, 2004). Thus, gene expression of catecholamine biosynthetic enzymes in both the sympathetic ganglia and the adrenal medulla is markedly elevated as part of the adaptation mechanism of the organism to long-term exposure to a homotypic stressor.

4.2. Transcription factors

4.2.1 Adrenal medulla

Transcription factors are part of a dynamic interplay that converts short-term transient activation of transcription to prolonged, potentially maladaptive

changes in gene expression (reviewed by Kvetnansky et al., 2009; Sabban & Kvetnansky, 2001; Sabban, Liu, Serova, Gueorguiev, & Kvetnansky, 2006; Sabban et al., 2012). The rapid activation of gene transcription for TH and DBH after 5 min of immobilization is too soon to reflect *de novo* synthesis of induced transcription factors. Increased phosphorylation of cAMP response element binding protein (CREB) occurs in the adrenal medulla of rats immobilized for only 5 min (Sabban, Nankova, et al., 2004). The activation of CREB at the CRE motif of the TH promoter is a well-characterized mechanism that is important for activation of TH gene transcription. Phosphorylation of a CREB serine residue needed for transcriptional activation begins after 5 min of immobilization and is more pronounced after 30 min. However, by 2 h of immobilization, levels of phosphorylated CREB returns to basal levels, reflecting the transient nature of this response. The phosphorylation of CREB on Ser-133 can be mediated by a number of kinases, including protein kinase A, mitogen activated protein kinases, and calmodulin dependent kinase.

After a single exposure to stress, the rise in mRNA levels is transient (Nankova et al., 1996; 1999). However, with a second exposure to the same stressor, there is already "memory" of the first experience such that the increase in TH mRNA is more prolonged. With prolonged repeated stress over 5–7 days, TH transcription remains high for as long as 2 days following cessation of stress. A single stress induces c-Fos in the adrenal medulla, which is correlated with its increased binding to the AP-1-like sites on the TH and DBH promoters (Nankova, Rivkin, Kelz, Nestler, & Sabban, 2000). The c-Fos is probably not the only AP-1 factor manifesting the second wave of gene expression in response to stressors. Fra-2 is not significantly changed during the first 30 min of stress. After 2 h, however, Fra-2 increases and is potentiated by repeated stress. This might be one of the mechanisms involved in permanently elevated TH mRNA levels in the adrenal medulla of repeatedly immobilized rats.

The immediate early gene Egr1 is also induced in rat adrenal medulla by a single stress exposure. Egr1 stimulates PNMT transcription in combination with glucocorticoids (Wong, Tai, Wong-Faull, Claycomb, & Kvetnansky, 2004). Egr1 also participates in cholinergic stimulation of the PNMT promoter. Reduced levels of Egr1 in the adrenal medulla of CRH knockout mice exposed to immobilization stress correlate well with reduced PNMT mRNA levels in those animals (Kvetnansky et al., 2006). Egr1 can also regulate transcription of the TH gene. Thus, a single transcription factor can stimulate catecholamine genes by more than one pathway and can stimulate more than one gene.

Following a single stress for 2 h, several hundred genes are significantly up- and downregulated (651 up- and 487 downregulated). With repeated stress, 370 genes were up- and 195 downregulated (Liu, Serova, Kvetnansky, & Sabban, 2008). A substantial number of gene changes were from the group of transcriptional factors and growth factor-related transcripts. Interestingly, an analysis of the direct interactions among the genes affected by stress indicates that Fos, Egr1, and nuclear receptor family member NR4A1 (also known as Nurr 77), which can interact with NBRE motif on the TH promoter, are likely to play a central role in interacting with many of the adrenomedullary genes regulated by a single immobilization stress. Repeated stress triggers an enormous induction of Fra-2 (Sabban et al., 2006), which is also phosphorylated with the repeated stress (Liu et al., 2005). The adrenal medulla does not appear to desensitize after repeated episodes of immobilization stress (even for 42 days); the final stressor still triggers induction of TH mRNA and Fos-related antigens such as Fra-2 (Kvetnansky, Jelokova, et al., 2002; Kvetnansky, Nankova, et al., 2002).

Stress also alters the expression of metabolism-, lipid-, protease-, and kinase/phosphatase-related genes. With repeated stress, a higher percentage of transcripts are devoted to neuropeptides. Most of the genes (>80%) altered by a single stress were transiently elevated. In contrast, approximately half of the genes elevated by repeated stress were also responsive to a single stress indicating that they mediate a prolonged response. There was also sustained phosphorylation of CREB throughout the entire course of repeated stress. Adrenal medullae of rats exposed to chronically repeated immobilization stress increased phosphorylated CREB 60-fold over unstressed controls.

Cold stress, like immobilization stress, triggered elevation of TH transcription in the adrenal medulla; however, the modified transcription factors were very different. Cold triggered increased binding to the AP-1 motif of the adrenal TH promoter, and a transient elevation of c-Fos was observed within the first few hours of cold exposure (Liu et al., 2005). However, there were no significant changes in phosphorylation of CREB or induction of Egr1. Fra-2 was also induced by cold stress, but the time course of changes differed from that observed with c-Fos. Levels of Fra-2 were not significantly changed with 1 or 7 days of cold stress but were about double basal levels after 28 days of continual cold stress (Liu et al., 2005). This is still a much smaller change than the changes in Fra-2 with repeated immobilization stress.

Microarray analysis reveals that immobilization and cold activate a very different repertoire of transcription factors in the adrenal medulla. Cold triggers significant changes in expression of fewer genes than immobilization with overlap in only about 5% of the genes up- or downregulated. Thus, there is specificity of response of the organism exposed to various stressors even at the molecular genetic level of expression of different genes. Animals preexposed to immobilization had a greater cold response in P-CREB, Egr1, and Fra-2. Rats preexposed to 28 days of cold displayed a significantly higher response to immobilization. Thus, different stressors can accentuate the molecular responses to each other.

4.2.2 Sympathetic ganglia

The mechanisms of stress-triggered activation of TH and DBH gene expression differ between sympathetic nerves and the adrenal medulla (reviewed by Sabban, 2007; Sabban, Nankova, et al., 2004). The most intriguing differences are in the response of TH and DBH gene expression to ACTH. ACTH can have a direct effect on expression of genes in sympathetic ganglia but not in the adrenal medulla (Kvetnansky et al., 2004). The marked induction of AP-1 factors, c-Fos, and Fra-2 observed in the adrenal medulla does not appear to be important in sympathetic ganglia (Kvetnansky et al., 2009; Sabban, Nankova, et al., 2004). Immobilization stress increased CREB binding at the CRE element on the TH gene promoter in the superior cervical ganglia (Sabban, Nankova, et al., 2004), suggesting increased expression of CREB and showing that related transcriptional mechanisms are likely involved. In contrast, in the adrenal medulla, the same stressor did not alter CREB levels, but rather triggered increased phosphorylation of CREB. Thus, induction of CREB might play a critical role in the stress response in the SCG, and this might mediate a sustained elevation of transcription of TH, DBH, and other CREB-responsive genes. The c-Jun N-terminal kinase is also selectively induced in the adrenal medulla, but not in the superior cervical ganglion.

The time course of stress-induced changes in mRNA levels is also different in sympathetic ganglia compared to the adrenal medulla. In stellate ganglia, the time course of the elevation of TH and DBH mRNA levels was more gradual and the extent of elevation more modest than in the adrenal medulla. In contrast, in the adrenal medulla, there was a rapid change in TH gene expression with only a single exposure to immobilization.

Although stress-induced PNMT gene expression is regulated by the HPA axis in both the adrenal medulla and sympathetic ganglia of rats and

mice (Kubovcakova et al., 2006; Kvetnansky et al., 2006), the involvement of the HPA axis in the stress-triggered increase in TH and DBH gene expression differs between the sympathetic ganglia and adrenal medulla. In summary, the sympathoneural system, at least as it relates to TH and DBH, is regulated distinctly from the adrenal medulla.

4.2.3 Regulation by the HPA axis

Trans-synaptic stimulation regulates catecholamine biosynthetic enzymes in sympathetic nerves. Nerve stimulation increased TH and DBH activities and elevated TH protein and mRNA levels in rat sympathetic ganglia. Denervation prevented the elevation of TH in response to reserpine. However, trans-synaptic inputs are not the only regulatory inputs. Decentralization greatly reduced basal TH activity, but exposure to cold stress triggered a large induction in TH activity in sympathetic ganglia even following decentralization.

Regulation of TH and DBH (in contrast to PNMT) gene expression in the adrenal medulla with stress is not very much affected by the HPA axis, and increased TH and DBH expression is still observed in hypophysectomized animals. However, the activation of the HPA axis by stress appears to be involved in the regulation of catecholamine biosynthetic enzymes in the sympathoneural system. A single dose of glucocorticoid increased TH activity and TH mRNA and enhanced the response to cold in sympathetic ganglia. Furthermore, injection of ACTH triggered a large rise in TH mRNA in superior cervical ganglia and enhanced expression of ACTH (MC2) receptor mRNA in the superior cervical and stellate ganglia (Nankova, Kvetnansky, & Sabban, 2003; Sabban, Nankova, et al., 2004). The effect of ACTH appears to occur, at least partially, by an adrenal-independent mechanism. Adrenalectomy, which, as expected, eliminated circulating E and increased plasma ACTH, also markedly raised plasma NE levels (Kvetnansky et al., 1995). Moreover, TH and DBH mRNAs in rat superior cervical ganglia are also elevated in adrenalectomized rats (Sabban et al., 2006; Serova, Gueorguiev, Cheng, & Sabban, 2008). These findings suggest that ACTH may have a direct effect on gene expression of catecholamine biosynthetic enzymes in sympathetic ganglia. Catecholamines also are involved in central nervous stimulation of ACTH release. The locus coeruleus has widespread NE projections, notably to the medial prefrontal cortex, which inhibits HPA responses to emotional stress. Injection of axonally transported immunotoxin to selectively ablate NE inputs to the prefrontal cortex diminished the stress-induced output of the HPA (Radley et al. 2008). Thus, central NE pathways can enhance the release

of ACTH in response to stress. This feed forward mechanism in which stress activates CNS catecholamine release of ACTH and ACTH activates peripheral catecholamine production has implications for stress-induced hypertension. In addition, these pathways might help explain the occurrence of human hypertension following administration of glucocorticoids such as dexamethasone that do not cause sodium retention.

Substantial evidence supports the importance of the activation of the HPA axis for regulation of PNMT gene expression. Hypophysectomy prevents the immobilization-triggered induction of PNMT gene expression. Glucocorticoids regulate PNMT in at least two ways: posttranscriptionally and transcriptionally (Wong & Tank, 2007). The PNMT promoter contains three glucocorticoid response elements (Tai, Claycomb, Her, Bloom, & Wong, 2002).The glucocorticoid receptor interacts with the PNMT promoter alone and also synergistically with Egr1 and AP-2 transcription factors. The HPA axis is also important in regulation of PNMT in the sympathoneural system. PNMT was detected in stellate ganglia and is elevated by immobilization stress in wild-type but not in CRH-deficient mice (Kubovcakova et al., 2006; Kvetnansky et al., 2006). A comparison of the regulation of gene expression for catecholamine biosynthetic enzymes in sympathetic ganglia with the adrenal medulla shows that in contrast to the original designation of the "sympathoadrenal" system, these are clearly two distinct systems—sympathoadrenomedullary and sympathoneural.

5. CONCLUSION

Stressors such as hypoglycemia, cold, and restraint selectively activate catecholamine release from sympathetic nerves and the adrenal medulla. Trans-synaptic stimulation induces catecholamine biosynthetic enzymes in nerves and the adrenal medulla by different pathways of CRE phosphorylation and expression factor synthesis. On the other hand, glucocorticoids are the primary stimulus to enzyme synthesis in nonneuronal catecholamine-producing cells. Because of these differences, stressors selectively activate different parts of peripheral catecholamine systems. However, chronic stress leads to longer-lasting elevations of catecholamine-synthesizing enzymes, and the adaptation to one stressor may potentiate the response to a single exposure of a different stressor.

Human studies indicate that chronic stress can lead to prolonged catecholamine increases and deleterious cardiovascular responses. Molecular studies in animals show that prolonged catecholamine activation following stress not

only is a consequence of central nervous memories but also reflects intracellular changes in the peripheral sympathetic nervous system. In addition, the additive effects of glucocorticoids, nerve traffic, and angiotensin II on catecholamine synthetic enzymes suggest potential therapeutic agents that can block these effects to reverse excess catecholamine release in patients with chronic stress. Rodent studies indicate that maternal stress sufficient to elevate glucocorticoid levels may induce hypertension in the offspring. We conclude that stress-induced changes in the peripheral sympathetic system are individualized and complex but comprehensible. These changes appear to have important cardiovascular consequences and probably play a role in stress-induced cardiac arrhythmias, hypertension, and the metabolic syndrome.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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REFERENCES

Armando, I., Jezova, M., Bregonzio, C., Baiardi, G., & Saavedra, J. M. (2004). Angiotensin II AT1 and AT2 receptor types regulate basal and stress-induced adrenomedullary catecholamine production through transcriptional regulation of tyrosine hydroxylase. *Annals of the New York Academy of Sciences*, *1018*, 302–309.

Bao, X., Lu, C. M., Liu, F., Gu, Y., Dalton, N. D., Zhu, B. Q., et al. (2007). Epinephrine is required for normal cardiovascular responses to stress in the phenylethanolamine N-methyltransferase knockout mouse. *Circulation*, *116*, 1024–1031.

Culman, J., Torda, T., Petrikova, M., & Murgas, K. (1988). Effect of corticosterone treatment and adrenalectomy on phenylethanolamine *N*-methyltransferase and catecholamines in brain stem and hypothalamic nuclei and superior cervical ganglion of rats. *Endocrinologia Experimentalis*, *22*, 117–128.

Dimsdale, J. E., & Moss, J. (1980). Plasma catecholamines in stress and exercise. *Journal of the American Medical Association*, *243*(4), 340–342.

Dobrakovova, M., Kvetnansky, R., Oprsalova, Z., & Jezova, D. (1993). Specificity of the effect of repeated handling on sympathetic-adrenomedullary and pituitary-adrenocortical activity in rats. *Psychoneuroendocrinology*, *18*, 163–174.

Dronjak, S., Ondriska, M., Svetlovska, D., Jezova, D., & Kvetnansky, R. (2002). Effects of novel stressors on plasma catecholamine levels in rats exposed to long-term cold. In R. McCarty, G. Aguilera, E. L. Sabban, & R. Kvetnansky (Eds.), *Stress neural, endocrine and molecular studies* (pp. 83–89). London: Taylor and Francis.

Eiden, L. E., Schutz, B., Anlauf, M., Depboylu, C., Schafer, M. K. H., & Weihe, E. (2002). The vesicular monoamine transporters (VMATs): Role in the chemical coding of neuronal transmission and monoamine storage in amine handling immune and inflammatory cells. In T. Nagatsu, T. Nabeshima, R. McCarty, & D. S. Goldstein (Eds.), *Catecholamine*

research: From molecular insights to clinical medicine (pp. 23–33). New York: Kluwer Academic/Plenum.
Eisenhofer, G. (2001). The role of neuronal and extraneuronal plasma membrane transporters in the inactivation of peripheral catecholamines. *Pharmacology & Therapeutics*, *91*, 35–62.
Eisenhofer, G., Cox, H. S., & Esler, M. D. (1990). Parallel increases in noradrenaline reuptake and release into plasma during activation of the sympathetic nervous system in rabbits. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *342*, 328–335.
Eisenhofer, G., Esler, M. D., Meredith, I. T., Ferrier, C., Lanbert, G., & Jennings, G. (1991). Neuronal re-uptake of noradrenaline by sympathetic nerves in humans. *Clinical Science*, *80*, 257–263.
Eisenhofer, G., Goldstein, D. S., & Kopin, I. J. (1989). Plasma dihydroxyphenylglycol for estimation of noradrenaline neuronal re-uptake in the sympathetic nervous system in vivo. *Clinical Science*, *76*, 171–182.
Eisenhofer, G., Keiser, H., Friberg, P., Mezey, E., Huynh, T. T., Hiremagalur, B., et al. (1998). Plasma metanephrines are markers of pheochromocytoma produced by catechol-*O*-methyltransferase within tumors. *Journal of Clinical Endocrinology and Metabolism*, *83*, 2175–2185.
Eisenhofer, G., Kopin, I. J., & Goldstein, D. S. (2004a). Catecholamine metabolism: A contemporary view with implications for physiology and medicine. *Pharmacological Reviews*, *56*, 331–349.
Eisenhofer, G., Kopin, I. J., & Goldstein, D. S. (2004b). Leaky catecholamine stores: Undue waste or a stress response coping mechanism? *Annals of the New York Academy of Sciences*, *1018*, 224–230.
Eisenhofer, G., & Lenders, J. W. (1998). Clues to the diagnosis of pheochromocytoma from the differential tissue metabolism of catecholamines. *Advances in Pharmacology*, *42*, 374–377.
Eisenhofer, G., Smolich, J. J., Cox, H. S., & Esler, M. D. (1991). Neuronal reuptake of norepinephrine and production of dihydroxyphenylglycol by cardiac sympathetic nerves in the anesthetized dog. *Circulation*, *84*, 1354–1363.
Elayan, H. H., Kennedy, B. P., & Ziegler, M. G. (1990). Cardiac atria and ventricles contain different inducible adrenaline synthesising enzymes. *Cardiovascular Research*, *24*, 53–56.
Esler, M., Jackman, G., Bobik, A., Kelleher, D., Jennings, G., Leonard, P., et al. (1979). Determination of norepinephrine apparent release rate and clearance in humans. *Life Sciences*, *25*, 1461–1470.
Fung, M. M., Peters, K., Redline, S., Ziegler, M. G., Ancoli-Israel, S., Barrett-Connor, E., et al. (2011). Decreased slow wave sleep increases risk of developing hypertension in elderly men. *Hypertension*, *58*, 596–603.
Goldstein, D. S. (1995). *Stress, catecholamines, and cardiovascular disease*. New York: Oxford University Press p. 539.
Goldstein, D. S. (2001). *The autonomic nervous system in health and disease*. New York: Marcel Bekker p. 618.
Goldstein, D. S. (2003). Catecholamines and stress. *Endocrine Regulations*, *37*, 69–80.
Goldstein, D. S. (2010). Catecholamines 101. *Clinical Autonomic Research*, *20*, 331–352.
Goldstein, D. S., & Holmes, C. (2008). Neuronal source of plasma dopamine. *Clinical Chemistry*, *54*, 1864–1871.
Goldstein, D. S., & Kopin, I. J. (2007). Evolution of concepts of stress. *Stress*, *10*, 109–120.
Goldstein, D. S., & Kopin, I. J. (2008). Adrenomedullary, adrenocortical, and sympathoneural responses to stressors: A meta-analysis. *Endocrine Regulations*, *42*, 111–119.
Goncalvesova, E., Micutkova, L., Mravec, B., Ksinantova, L., Krizanova, O., Fabian, J., et al. (2004). Changes in gene expression of phenylethanolamine *N*-methyltransferase in the transplanted human heart. *Annals of the New York Academy of Sciences*, *1018*, 430–436.

Gorbunova, A. V., & Kashtanov, S. I. (1983). Monoamine oxidase activity in autonomic ganglia of rabbits with differing resistance of cardiovascular functions to emotional stress. *Biulleten' Eksperimental'noĭ Biologii i Meditsiny*, *96*, 111–113.

Huang, M. H., Friend, D. S., Sunday, M. E., Singh, K., Haley, K., Austen, K. F., et al. (1996). An intrinsic adrenergic system in mammalian heart. *The Journal of Clinical Investigation*, *98*, 1298–1303.

Iversen, L. (1965). The uptake of catecholamines at high perfusion concentrations in the rat isolated heart: A novel catecholamine uptake process. *British Journal of Pharmacology*, *25*, 18–33.

Jabbi, M., Kema, I. P., van der Pompe, G., Meerman, G. J., Ormel, J., & den Boer, J. A. (2007). Catechol-o-methyltransferase polymorphism and susceptibility to major depressive disorder modulates psychological stress response. *Psychiatric Genetics*, *17*, 183–193.

Jelokova, J., Rusnak, M., Kubovcakova, L., Buckendahl, P., Krizanova, O., Sabban, E. L., et al. (2002). Stress increases gene expression of phenylethanolamine *N*-methyltransferase in spleen of rats via pituitary-adrenocortical mechanism. *Psychoneuroendocrinology*, *27*, 619–633.

Johnson, G. R. J. (1988). Accumulation of biological amines into chromaffin granules: A model for hormone and neurotransmitter transport. *Physiological Reviews*, *68*, 232–307.

Kennedy, B., Bigby, T. D., & Ziegler, M. G. (1995). Nonadrenal epinephrine-forming enzymes in humans. Characteristics, distribution, regulation, and relationship to epinephrine levels. *The Journal of Clinical Investigation*, *95*(6), 2896–2902.

Kennedy, B., Elayan, H., & Ziegler, M. G. (1993a). Glucocorticoid elevation of mRNA encoding epinephrine-forming enzyme in lung. *American Journal of Physiology. Lung Cellular and Molecular Physiology*, *265*, L117–L120.

Kennedy, B., Elayan, H., & Ziegler, M. G. (1993b). Glucocorticoid hypertension and nonadrenal phenylethanolamine *N*-methyltransferase. *Hypertension*, *21*, 415–419.

Kennedy, B., & Ziegler, M. G. (1991). Cardiac epinephrine synthesis. Regulation by a glucocorticoid. *Circulation*, *84*(2), 891–895.

Kiran, B. K., & Ulus, I. H. (1992). Selective response of rat peripheral sympathetic nervous system to various stress situations. In R. Kvetnansky, R. McCarty, & J. Axelrod (Eds.), *Stress: Neuroendocrine and molecular approaches* (pp. 561–568). New York: Gordon and Breach.

Kiss, A., Mravec, B., Palkovits, M., & Kvetnanský, R. (2008). Stress-induced changes in tyrosine hydroxylase gene expression in rat hypothalamic paraventricular, periventricular, and dorsomedial nuclei. *Annals of the New York Academy of Sciences*, *1148*, 74–85.

Kobayashi, K., & Nagatsu, T. (2005). Molecular genetics of tyrosine 3-monooxygenase and inherited diseases. *Biochemical and Biophysical Research Communications*, *338*, 267–270.

Krizanova, O., Micutkova, L., Jelokova, J., Filipenko, M., Sabban, E., & Kvetnansky, R. (2001). Existence of cardiac PNMT mRNA in adult rats: Elevation by stress in a glucocorticoid-dependent manner. *American Journal of Physiology. Heart and Circulatory Physiology*, *281*, H1372–H1379.

Kubovcakova, L., Micutkova, L., Bartosova, Z., Sabban, E. L., Krizanova, O., & Kvetnansky, R. (2006). Identification of phenylethanolamine *N*-methyltransferase gene expression in stellate ganglia and its modulation by stress. *Journal of Neurochemistry*, *97*, 1419–1430.

Kvetnansky, R. (2004). Stressor specificity and effect of prior experience on catecholamine biosynthetic enzyme phenylethanolamine *N*-methyltransferase. *Annals of the New York Academy of Sciences*, *1032*, 117–129.

Kvetnansky, R., Albrecht, I., Torda, T., Saleh, N., Jahnova, E., & Mikulaj, L. (1976). Effect of stress on catecholamine synthesizing and degrading enzymes in control and

spontaneously hypertensive rats. In E. Usdin, R. Kvetnansky, & I. J. Kopin (Eds.), *Catecholamines and stress* (pp. 237–249). Oxford: Pergamon.

Kvetnansky, R., Armando, I., Weise, V. K., Holmes, C., Fukuhara, K., Deka-Starosta, A., et al. (1992). Plasma dopa responses during stress: Dependence on sympathoneural activity and tyrosine hydroxylation. *Journal of Pharmacology and Experimental Therapeutics*, *261*, 899–909.

Kvetnansky, R., Goldstein, D. S., Weise, V. K., Holmes, C., Szemeredi, K., Bagdy, G., et al. (1992). Effects of handling or immobilization on plasma levels of 3,4-dihydroxyphenylalanine, catecholamines, and metabolites in rats. *Journal of Neurochemistry*, *58*, 2296–2302.

Kvetnansky, R., Jelokova, J., Rusnak, M., Dronjak, S., Serova, L., Nankova, B., et al. (2002). Novel stressors exaggerate tyrosine hydroxylase gene expression in the adrenal medulla of rats exposed to long-term cold stress. In R. McCarty, G. Aguilera, E. L. Sabban, & R. Kvetnansky (Eds.), *Stress neural, endocrine and molecular studies* (pp. 121–128). New York: Taylor and Francis.

Kvetnansky, R., Kubovcakova, L., Tillinger, A., Micutkova, L., Krizanova, O., & Sabban, E. L. (2006). Gene expression of phenylethanolamine *N*-methyltransferase in corticotropin-releasing hormone knockout mice during stress exposure. *Cellular and Molecular Neurobiology*, *26*, 733–752.

Kvetnansky, R., & McCarty, R. (2007). Adrenal medulla. In G. Fink (Ed.), *Encyclopedia of stress* (pp. 52–59). New York: Academic.

Kvetnansky, R., Micutkova, L., Rychkova, N., Kubovcakova, L., Mravec, B., Filipenko, M., et al. (2004). Quantitative evaluation of catecholamine enzymes gene expression in adrenal medulla and sympathetic ganglia of stressed rats. *Annals of the New York Academy of Sciences*, *1018*, 356–369.

Kvetnansky, R., & Mikulaj, L. (1970). Adrenal and urinary catecholamines in rats during adaptation to repeated immobilization stress. *Endocrinology*, *87*, 738–743.

Kvetnansky, R., Nankova, B., Rusnak, M., Micutkova, L., Kubovcakova, L., Dronjak, S., et al. (2002). Differential gene expression of tyrosine hydroxylase in rats exposed long-term to various stressors. In T. Nagatsu, T. Nabeshima, R. McCarty, & D. S. Goldstein (Eds.), *Catecholamine research: From molecular insights to clinical medicine* (pp. 317–320). New York: Plenum.

Kvetnansky, R., Nemeth, S., Vigas, M., Oprsalova, Z., & Jurcovicova, J. (1984). Plasma catecholamines in rats during adaptation to intermittent exposure to different stressors. In E. Usdin, R. Kvetnansky, & J. Axelrod (Eds.), *Stress: The role of catecholamines and other neurotransmitters* (pp. 537–562). New York: Gordon and Breach Science.

Kvetnansky, R., Pacak, K., Fukuhara, K., Viskupic, E., Hiremagalur, B., Nankova, B., et al. (1995). Sympathoadrenal system in stress. Interaction with the hypothalamic-pituitary-adrenocortical system. *Annals of the New York Academy of Sciences*, *771*, 131–158.

Kvetnansky, R., Pacak, K., Sabban, E. L., Kopin, I. J., & Goldstein, D. S. (1998). Stressor specificity of peripheral catecholaminergic activation. *Advances in Pharmacology*, *42*, 556–560.

Kvetnansky, R., & Sabban, E. L. (1993). Stress-induced changes in tyrosine hydroxylase and other catecholamine biosynthetic enzymes. In M. Naoi & S. H. Parvez (Eds.), *Tyrosine hydroxylase: From discovery to cloning*. Utrecht: VSP.

Kvetnansky, R., & Sabban, E. L. (1998). Stress and molecular biology of neurotransmitter-related enzymes. *Annals of the New York Academy of Sciences*, *851*, 342–356.

Kvetnansky, R., Sabban, E. L., & Palkovits, M. (2009). Catecholaminergic systems in stress: Structural and molecular genetic approaches. *Physiological Reviews*, *89*, 535–606.

Kvetnansky, R., Sun, C. L., Lake, C. R., Thoa, N., Torda, T., & Kopin, I. J. (1978). Effect of handling and forced immobilization on rat plasma levels of epinephrine, norepinephrine, and dopamine-beta-hydroxylase. *Endocrinology*, *103*, 1868–1874.

Kvetnansky, R., Torda, T., Jahnova, E., & Saleh, N. (1975). Activity of catecholamine degrading enzymes in rat adrenal medulla and cortex after acute and repeated stress. *Endocrinologia Experimentalis*, *9*, 79–86.

Kvetnansky, R., Torda, T., Macho, L., Tigranian, R. A., Serova, L., & Genin, A. M. (1981). Effect of weightlessness on sympathetic-adrenomedullary activity of rats. *Acta Astronautica*, *8*, 469–481.

Kvetnansky, R., Ukropec, J., Laukova, M., Manz, B., Pacak, K., & Vargovic, P. (2012). Stress stimulates production of catecholamines in rat mesenteric adipocytes. *Cellular and Molecular Neurobiology*, *32*, 801–813.

Kvetnansky, R., Weise, V. K., & Kopin, I. J. (1970). Elevation of adrenal tyrosine hydroxylase and phenylethanolamine-*N*-methyl transferase by repeated immobilization of rats. *Endocrinology*, *87*, 744–749.

Kvetnansky, R., Weise, V. K., Thoa, N. B., & Kopin, I. J. (1979). Effects of chronic guanethidine treatment and adrenal medullectomy on plasma levels of catecholamines and corticosterone in forcibly immobilized rats. *Journal of Pharmacology and Experimental Therapeutics*, *209*, 287–291.

Lachman, H. M., Nolan, K. A., Mohr, P., Saito, T., & Volavka, J. (1998). Association between catechol *O*-methyltransferase genotype and violence in schizophrenia and schizoaffective disorder. *The American Journal of Psychiatry*, *155*, 835–837.

Lemoine, A. P., Armando, I., Brun, J. C., Segura, E. T., & Barontini, M. (1990). Footshock affects heart and brain MAO and MAO inhibitory activity and open field behavior in rats. *Pharmacology, Biochemistry, and Behavior*, *36*, 85–88.

Levitt, N. S., Lindsay, R. S., Holmes, M. C., & Seckl, J. R. (1996). Dexamethasone in the last week of pregnancy attenuates hippocampal glucocorticoid receptor gene expression and elevates blood pressure in the adult offspring in the rat. *Neuroendocrinology*, *64*, 412–418.

Liu, X., Kvetnansky, R., Serova, L., Sollas, A., & Sabban, E. L. (2005). Increased susceptibility to transcriptional changes with novel stressor in adrenal medulla of rats exposed to prolonged cold stress. *Molecular Brain Research*, *141*, 19–29.

Liu, X., Serova, L., Kvetnansky, R., & Sabban, E. L. (2008). Identifying the stress transcriptome in the adrenal medulla following acute and repeated immobilization. *Annals of the New York Academy of Sciences*, *1148*, 1–28.

McEwen, B. S. (1998). Stress, adaptation, and disease. Allostasis and allostatic load. *Annals of the New York Academy of Sciences*, *840*, 33–44.

McEwen, B. S. (2004). Protection and damage from acute and chronic stress: Allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. *Annals of the New York Academy of Sciences*, *1032*, 1–7.

Mezey, E., Eisenhofer, G., Hansson, S., Harta, G., Hoffman, B. J., Gallatz, K., et al. (1999). Non-neuronal dopamine in the gastrointestinal system. *Clinical and Experimental Pharmacology & Physiology. Supplement*, *26*, S14–S22.

Mezey, E., Eisenhofer, G., Harta, G., Hansson, S., Gould, L., Hunyady, B., et al. (1996). A novel nonneuronal catecholaminergic system: Exocrine pancreas synthesizes and releases dopamine. *Proceedings of the National Academy of Sciences of the United States of America*, *93*, 10377–10382.

Micutkova, L., Rychkova, N., Sabban, E. L., Krizanova, O., & Kvetnansky, R. (2003). Quantitation of changes in gene expression of norepinephrine biosynthetic enzymes in rat stellate ganglia induced by stress. *Neurochemistry International*, *43*, 235–242.

Mikhailova, O. N., Gulyaeva, L. F., & Filipenko, M. L. (2005). Gene expression of drug metabolizing enzymes in adult and aged mouse liver: A modulation by immobilization stress. *Toxicology*, *210*, 189–196.

Nankova, B., Kvetnansky, R., Hiremagalur, B., Sabban, B., Rusnak, M., & Sabban, E. L. (1996). Immobilization stress elevates gene expression for catecholamine biosynthetic

enzymes and some neuropeptides in rat sympathetic ganglia: Effects of adrenocorticotropin and glucocorticoids. *Endocrinology*, *137*, 5597–5604.

Nankova, B. B., Kvetnansky, R., & Sabban, E. L. (2003). Adrenocorticotropic hormone (MC-2) receptor mRNA is expressed in rat sympathetic ganglia and up-regulated by stress. *Neuroscience Letters*, *344*, 149–152.

Nankova, B. B., Rivkin, M., Kelz, M., Nestler, E. J., & Sabban, E. L. (2000). Fos related antigen 2: Potential mediator of the transcriptional activation in rat adrenal medulla evoked by repeated immobilization stress. *Journal of Neuroscience*, *20*, 5647–5653.

Nankova, B. B., & Sabban, E. L. (1999). Multiple signalling pathways exist in the stress-triggered regulation of gene expression for catecholamine biosynthetic enzymes and several neuropeptides in the rat adrenal medulla. *Acta Physiologica Scandinavica*, *167*, 1–9.

Nankova, B. B., Tank, A. W., & Sabban, E. L. (1999). Transient or sustained transcriptional activation of the genes encoding rat adrenomedullary catecholamine biosynthetic enzymes by different durations of immobilization stress. *Neuroscience*, *94*, 803–808.

Obata, T., & Yamanaka, Y. (1994). Changes in monoamine oxidase activity in rat liver during stress. *Japanese Journal of Pharmacology*, *66*, 149–150.

Pacak, K., & Palkovits, M. (2001). Stressor specificity of central neuroendocrine responses: Implications for stress-related disorders. *Endocrine Reviews*, *22*, 502–548.

Pacak, K., Palkovits, M., Yadid, G., Kvetnansky, R., Kopin, I. J., & Goldstein, D. S. (1998). Heterogeneous neurochemical responses to different stressors: A test of Selye's doctrine of nonspecificity. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *275*, R1247–R1255.

Paivarinta, H., Pickel, V. M., Eranko, L., & Joh, T. H. (1989). Glucocorticoid induced PNMT-immunoreactive sympathetic cells in the superior cervical ganglion of the rat. *Journal of Electron Microscopy Technique*, *12*, 389–396.

Radley, J. J., Williams, B., & Sawchenko, P. E. (2008). Noradrenergic innervation of the dorsal medial prefrontal cortex modulates hypothalamo-pituitary-adrenal responses to acute emotional stress. *Journal of Neuroscience*, *28*(22), 5806–5816.

Ramanathan, R., & Cryer, P. E. (2011). Adrenergic mediation of hypoglycemia-associated autonomic failure. *Diabetes*, *60*(2), 602–606.

Sabban, E. L. (2007). Catecholamines in stress: Molecular mechanisms of gene expression. *Endocrine Regulations*, *41*, 61–73.

Sabban, E. L., Hebert, M. A., Liu, X., Nankova, B., & Serova, L. (2004). Differential effects of stress on gene transcription factors in catecholaminergic systems. *Annals of the New York Academy of Sciences*, *1032*, 130–140.

Sabban, E. L., Hiremagalur, B., Nankova, B., & Kvetnansky, R. (1995). Molecular biology of stress-elicited induction of catecholamine biosynthetic enzymes. *Annals of the New York Academy of Sciences*, *771*, 327–338.

Sabban, E. L., & Kvetnansky, R. (2001). Stress-triggered activation of gene expression in catecholaminergic systems: Dynamics of transcriptional events. *Trends in Neurosciences*, *24*, 91–98.

Sabban, E. L., Liu, X., Serova, L., Gueorguiev, V., & Kvetnansky, R. (2006). Stress triggered changes in gene expression in adrenal medulla: Transcriptional responses to acute and chronic stress. *Cellular and Molecular Neurobiology*, *26*, 843–854.

Sabban, E. L., Nankova, B. B., Serova, L. I., Kvetnansky, R., & Liu, X. (2004). Molecular regulation of gene expression of catecholamine biosynthetic enzymes by stress: Sympathetic ganglia versus adrenal medulla. *Annals of the New York Academy of Sciences*, *1018*, 370–377.

Sabban, E. L., Tillinger, A., Nostramo, R., & Serova, L. (2012). Stress triggered changes in expression of gene for neurosecretory granules in adrenal medulla. *Cellular and Molecular Neurobiology*, *32*, 795–800.

Saygili, E., Günzel, C., Saygili, E., Noor-Ebad, F., Schwinger, R. H., Mischke, K., et al. (2011). Irregular electrical activation of intrinsic cardiac adrenergic cells increases catecholamine-synthesizing enzymes. *Biochemical and Biophysical Research Communications*, *413*, 432–435.

Schalling, M., Franco-Cereceda, A., Hemsen, A., Dagerlind, A., Seroogy, K., Persson, H., et al. (1991). Neuropeptide Y and catecholamine synthesizing enzymes and their mRNAs in rat sympathetic neurons and adrenal glands: Studies on expression, synthesis and axonal transport after pharmacological and experimental manipulations using hybridization techniques and radioimmunoassay. *Neuroscience*, *41*, 753–766.

Schuldiner, S. (1994). A molecular glimpse of vesicular monoamine transporters. *Journal of Neurochemistry*, *62*, 2067–2078.

Serova, L. I., Gueorguiev, V., Cheng, S. Y., & Sabban, E. L. (2008). Adrenocorticotropic hormone elevates gene expression for catecholamine biosynthesis in rat superior cervical ganglia and locus coeruleus by an adrenal independent mechanism. *Neuroscience*, *153*, 1380–1389.

Shaw, W. S., Patterson, T. L., Ziegler, M. G., Dimsdale, J. E., Semple, S. J., & Grant, I. (1999). Accelerated risk of hypertensive blood pressure recordings among Alzheimer caregivers. *Journal of Psychosomatic Research*, *46*, 215–227.

Singh, R. R., Cullen-McEwen, L. A., Kett, M. M., Boon, W. M., Dowling, J., Bertram, J. F., et al. (2007). Prenatal corticosterone exposure results in altered AT1/AT2, nephron deficit, and hypertension in the rat offspring. *The Journal of Physiology*, *579*(Pt 2), 503–513.

Slavikova, J., Kuncova, J., Reischig, J., & Dvorakova, M. (2003). Catecholaminergic neurons in the rat intrinsic cardiac nervous system. *Neurochemical Research*, *28*, 593–598.

Smolka, M. N., Schumann, G., Wrase, J., Grusser, S. M., Flor, H., Mann, K., et al. (2005). Catechol-*O*-methyltransferase val158met genotype affects processing of emotional stimuli in the amygdala and prefrontal cortex. *Journal of Neuroscience*, *25*, 836–842.

Stone, E. A., & McCarty, R. (1983). Adaptation to stress: Tyrosine hydroxylase activity and catecholamine release. *Neuroscience and Biobehavioral Reviews*, *7*, 29–34.

Tai, T. C., Claycomb, R., Her, S., Bloom, A. K., & Wong, D. L. (2002). Glucocorticoid responsiveness of the rat phenylethanolamine N-methyltransferase gene. *Molecular Pharmacology*, *61*, 1385–1392.

Tillinger, A., Novakova, M., Pavlovicova, M., Lacinova, L., Zatovicova, M., Pastorekova, S., et al. (2006). Modulation by 6-hydroxydopamine of expression of the phenylethanolamine *N*-methyltransferase (PNMT) gene in the rat heart during immobilization stress. *Stress*, *9*, 207–213.

Tillinger, A., Sollas, A., Serova, L. I., Kvetnansky, R., & Sabban, E. L. (2010). Vesicular monoamine transporters (VMAT) in adrenal chromaffin cells: Stress-triggered induction of VMAT2 and expression in epinephrine synthesizing cells. *Cellular and Molecular Neurobiology*, *30*, 1459–1464.

Vargovic, P., Ukropec, J., Laukova, M., Cleary, S., Manz, B., Pacak, K., et al. (2011). Adipocytes as a new source of catecholamine production. *FEBS Letters*, *585*, 2279–2284.

Vargovic, P., Ukropec, J., Laukova, M., Kurdiova, T., Balaz, M., Manz, B., et al. (2013). Repeated immobilization stress induces catecholamine production in rat mesenteric adipocytes. *Stress*, *16*(3), 340–352.

von Känel, R., Ancoli-Israel, S., Dimsdale, J. E., Mills, P. J., Mausbach, B. T., Ziegler, M. G., et al. (2010). Sleep and biomarkers of atherosclerosis in elderly Alzheimer caregivers and controls. *Gerontology*, *56*, 41–50.

von Känel, R., Mausbach, B. T., Dimsdale, J. E., Mills, P. J., Patterson, T. L., Ancoli-Israel, S., et al. (2011). Cardiometabolic effects in caregivers of nursing home placement and death of their spouse with Alzheimer's disease. *Journal of American Geriatrics Society*, *9*, 2037–2044.

Weinshilboum, R. M., Kvetnansky, R., Axelrod, J., & Kopin, I. J. (1971). Elevation of serum dopamine-beta-hydroxylase activity with forced immobilization. *Nature: New Biology*, *230*, 287–288.

Wittstein, I. S. (2012). Stress cardiomyopathy: A syndrome of catecholamine-mediated myocardial stunning? *Cellular and Molecular Neurobiology*, *32*, 847–857.

Wolfovitz, E., Grossman, E., Folio, C. J., Keiser, H. R., Kopin, I. J., & Goldstein, D. S. (1993). Derivation of urinary dopamine from plasma dihydroxyphenylalanine in humans. *Clinical Science*, *84*, 549–557.

Wong, D. L., Lesage, A., Siddall, B., & Funder, J. W. (1992). Glucocorticoid regulation of phenylethanolamine N-methyltransferase in vivo. *The FASEB Journal*, *6*, 3310–3315.

Wong, D. L., Tai, T. C., Wong-Faull, D. C., Claycomb, R., & Kvetnansky, R. (2004). Genetic mechanisms for adrenergic control during stress. *Annals of the New York Academy of Sciences*, *1018*, 387–397.

Wong, D. L., & Tank, A. W. (2007). Stress-induced catecholaminergic function: Transcriptional and post-transcriptional control. *Stress*, *10*, 121–130.

Ziegler, M. G., Bao, X., Kennedy, B. P., Joyner, A., & Enns, R. (2002). Location, development, control, and function of extra-adrenal phenylethanolamine *N*-methyltransferase. *Annals of the New York Academy of Sciences*, *971*, 76–82.

Ziegler, M. G., Elayan, H., Milic, M., Sun, P., & Gharaibeh, M. (2012). Epinephrine and the metabolic syndrome. *Current Hypertension Reports*, *14*, 1–7.

Ziegler, M. G., Kennedy, B. P., & Houts, F. W. (1998). Extra-adrenal nonneuronal epinephrine and phenylethanolamine-*N*-methyltransferase. *Advances in Pharmacology*, *42*, 843–846.

Ziegler, M. G., Milic, M., & Elayan, H. (2011). Cardiovascular regulation in obstructive sleep apnea. *Drug Discovery Today: Disease Models*, *8*, 155–160.

Ziegler, M. G., Milic, M., Sun, P., Tang, Ch.-M., Elayan, H., Bao, X., et al. (2011). Endogenous epinephrine protects against obesity induced insulin resistance. *Autonomic Neuroscience*, *162*, 32–34.

Zubieta, J. K., Heitzeg, M. M., Smith, Y. R., Bueller, J. A., Xu, K., Xu, Y., et al. (2003). COMT val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. *Science*, *299*, 1240–1243.

Zukowska-Grojec, Z. (1995). Neuropeptide Y. A novel sympathetic stress hormone and more. *Annals of the New York Academy of Sciences*, *771*, 219–233.