

BioSonics, Stress Science, and Nitric Oxide¹Literature Review

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BioSonic research conducted at Cell Dynamics, Inc. demonstrates the release of Nitric Oxide (NO) from various tissue sources. Utilizing an amperometric detection system, allowing only nitric oxide to generate an upward deflection, we measure real-time NO release from living cells and demonstrate that the use of the BioSonic Otto™ (128cps), OM Tuner™ (136.1cps) and Body Tuners™ (C256 & G384) tuning forks have the ability to release NO well above baseline pulsatile levels. The release of NO is visualized as a large peak of pure nitric oxide immediately following exposure of these tissues to the vibration / sound of the tuning forks. Once NO is released it follows a well characterized signaling cascade, exhibiting established properties of NO. The next sections of this paper delineate the signaling cascade in tissues, emphasizing the biomedical significance of generating enhanced NO levels for a short duration.

Stress

Stress has been the focus of science, research, and practical medicine for many decades [1-4]. Stress as a concept describes the effects of psychosocial and environmental factors on physical or mental well-being [1,4,5]. Hence, stress implies a challenge (stimulus) that requires behavioral, psychological, and physiological changes (adaptations) to be successfully met, and a state of hyperarousal for the initiation of necessary counteracting reactions (stress response) [6-8]. Furthermore, 'balance' becomes important for the stress concept: Through an extremely complicated equilibrium called 'homeostasis', all living organisms maintain their survival in the face of both externally and internally generated stimuli (stressors). This apparent harmony is constantly challenged [9,10]. Thus, all life forms have developed mechanisms to overcome immediate perturbations, i.e., protective perturbation response [11]. As a result, 'allostasis' (a state of dynamic balance) may be achieved [6,7,12]. In this regard, an organism has to pay a price (i.e., energy, in particular) for repeatedly adapting to physical challenges and psychosocial threats. 'Allostatic load' refers to this cost of constantly adapting to repeated/ chronic environmental challenge and experiencing of fluctuating or heightened neuroendocrine response patterns over and over in response to stressors [13-15].

Two of the well-known molecules that play a major role in the allostatic stress response, each represents one 'arm' of the response (the hypothalamic-pituitary-adrenal (HPA) axis and the sympathoadrenal medullary (SAM) system [16]), are cortisol and norepinephrine (NE)/ epinephrine [16-19]. More recently, other molecules involved have been detected, e.g., melatonin [20] and anandamide [21], and the connection of nitric oxide with the stress response has also been proposed [21-25]. Hence, the objective of this paper is to elucidate common pathophysiological patterns of stress-related disease processes,

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particularly with regard to nitric oxide-coupled molecular pathways, and to examine the analogies found in these potentially overlapping underlying mechanisms.

Nitric oxide

Nitric oxide (NO) is a free radical that is constantly produced/ released throughout the body by diverse tissues, i.e., endothelium [22,23]. Thereby, it presumably is part of numerous (patho)physiological processes and pathways. Yet, the concrete role of NO in stress-related diseases represents an interesting field of investigation, since NO is involved in the stress physiology and apparently also takes part in stress-related disease processes [21,23-25]. Hence, the role of NO in specific stress-related diseases will be discussed.

Like stress, NO seems to be capable of principally exerting either beneficial/ ameliorating or deleterious effects [6,7,11,13,22,23]. The actual distinction depends on a multitude of factors, such as duration of (an enhanced) NO release, amount of produced NO, and type of synthesis of NO molecules [21-26]. Moreover, NO counteracts NE activity and sympathetic responsivity [21-24,26,27]. Additionally, NO inhibits the release of other monoamine transmitter molecules, e.g., dopamine [28], and here, autoregulatory pathways that involve different signaling molecules (like opiates and endocannabinoids) are implicated [21,26,28]. Thus, NO and the stress (patho)physiology are closely connected and molecular mechanisms or pathways may be shared, i.e., analogous, under certain conditions. Clearly, NO plays a significant role in several disease-associated processes.

NO is produced via two different mechanisms. Immediate release of NO is of constitutive nitric oxide synthase (cNOS) origin [22]. Thereby, cNOS is a calcium-dependent enzyme (reliant on intracellular calcium transients) that is constitutively and permanently expressed, in endothelial (eNOS), neuronal (nNOS), and immune cells, and produces NO at a low levels. This 'basal' NO can be increased for a short time via additional cNOS stimulation in response to certain signals [26]. The brief 'extra' cNOS-NO boost, though only in the nano-molar range, can exert lasting and profound physiological actions, still evident after NO returned to basal levels [26]. Hence, cNOS-derived NO release is part of acute response mechanisms that occur in many biological states [21,22,23,24,26,29]. In contrast, the inducible nitric oxide synthase (iNOS) is a calcium-independent enzyme that is prevalent in many tissues, yet only expressed 'on demand' in specific situations and under the influence of various signaling molecules, i.e., proinflammatory cytokines [26]. Following its induction, iNOS produces NO at higher levels (in the micro-molar range) after a latency period, and this NO release lasts for an extended period of time, i.e., days [26].

There obviously exists a close interdependency between the two different types of NO production (cNOS or iNOS-related). For example, cNOS inhibits/ balances iNOS activity [21,30]. Moreover, NO may actually represent a 'double-edged sword' [31], since small quantities produced by constitutive enzymes may predominantly mediate physiological effects, whereas the expression of iNOS may lead to larger quantities of NO, a situation that may be associated with cytotoxic and detrimental effects of NO observed in various disorders - if induced under inappropriate circumstances [31]. Thus, today, many beneficial effects of NO (especially cNOS-derived NO) have been described in the

literature - but, in parallel, the significance of NO for negative pathophysiological states and disease processes is also known (overview in: [29,32,33]). In this regard, there also exists confusion in the literature as to which form of NOS individuals are working with. Therefore, NO apparently has the potential to exert 'good' or 'bad', ameliorating or detrimental effects on health/ disease outcome, and the specific difference of activity may reflect a distinct type and amount of NO release, different affected disease states (specific points in time where NO action sets in and becomes vital for the further development), severity of disease, and varying capabilities of organisms to balance, shift, and terminate the underlying molecular pathways.

In more severe or chronic states of diseases, a more rigid and non-flexible regimen may have taken over. NO may be involved, but here, the detrimental effects of NO may play a more significant role than the ameliorating capacities. In contrast, in less rigid, less severe, and earlier states of diseases (or stressful situations), flexibility of biological processes may still be possible to a greater degree, and NO effects may predominantly be helpful. Yet, when chronic stress or an overwhelming acute stressor/ stimulus occurs (and in more advanced, severe disease states, or when an underlying predisposition comes into play), a loss of balance/ control may lead to more deleterious processes - or even to a disease-promoting vicious cycle.

Nitric Oxide and Stress-related diseases

A. Immunological diseases

Stress (stressors and stress responses) plays a major role in immunological diseases and immune-related disease processes. Inflammation, infection, autoimmune processes, and perhaps even the onset and development of malignant tumors may occasionally be associated with the stress phenomenon [13]. Thereby, it is widely accepted that acute stress tends to enhance immune functioning, whereas chronic stress more likely suppresses it [32]. However, the effects triggered by stress can be beneficial for some types of immune responses - and deleterious for others [33,34,35,36]. Thus, stress may represent a modulator of the immune or inflammatory response, whose outcome depends on a multitude of factors [34,35]. A crucial participatory component of importance, however, may be characterized by NO and its related (patho)physiological pathways.

Nitric oxide is known to have antimicrobial and tumoricidal properties [37]. Thereby, all known NOS isoforms operate in the immune system (including the thymus gland) [32,37]. NO activity has primarily been linked to antiinflammatory and immune suppressive effects - due to its predominant suppressive influence on inflammatory cytokine synthesis [26] -, but it may also be part of proinflammatory pathways [26]. As a reactive gas molecule (in its natural form) or in connection with carriers, NO can exert actions at distant or local sites concomitantly [32]. In doing so, the free radical has many reaction partners: NO interacts with DNA, regulatory molecules, proteins/ thiols, reactive oxygen intermediates, and prosthetic groups (as in heme) [32]. In the immune system, NO additionally is enzymatically connected to T and B lymphocytes [32].

The immune defense against pathogens often requires NO [38]. For example, iNOS has been reported to regulate the innate immune response, in part, by affecting natural killer (NK) cell function [38]. The production of NO during this innate immune response has

already been shown, and this process is potentiated by interferon-gamma (IFN-gamma), produced by NK and T cells [38]. As demonstrated for NE in acute stress, NO also helps to defend the body against invaders and to silence a parallel inflammation - e.g., by influencing the patterns of T cell activity (and cytokine production) [38]. Thus, NO is a frequent, beneficial regulatory component of defense.

However, NO is an essential cytotoxic agent in host defense itself, yet can be autotoxic if overproduced (as evidenced by inflammatory lesions and tissue destruction in experimental arthritis models coupled to NO production) [39]. The increased expression of iNOS and (over)generation of NO has been associated with the pathogenesis of chronic inflammation [39]. Nevertheless, in a recent study, a selective iNOS inhibition surprisingly exacerbated the chronic inflammatory response in experimentally induced arthritis in rats, and a distinct pattern of eNOS and nNOS expression in the inflamed arthritic synovium occurred. Thus, a selective inhibition of iNOS may actually worsen erosive joint disease, showing that iNOS also seems to have protective functions, while the constitutive NOS isoforms appear to even contribute to the evolution of - at least some - acute and chronic inflammatory pathology (in this animal model) [39]. Therefore, the common notion that inducible NO pathways are generally detrimental, whereas constitutive forms are ameliorating may not be supported here. Additional studies are required to better elucidate the complex nature of NO production in inflammatory diseases.

The nuclear (transcription) factor kappa B (NF-kappaB) is involved in activation and production of proinflammatory cytokines [26,40]. Further, the inhibitor of NF-kappaB kinase beta (IkappaB kinase beta or IKK beta) is a key regulator of NF-kappaB activity, wherefore its constitutive activation coincides with enhanced NF-kappaB activity and causes/ exacerbates chronic inflammatory diseases as rheumatoid arthritis (RA) [40]. IKK beta parallels NF-kappaB, and in principle, it can have both pro- and antiinflammatory effects [40]. However, its increased activity is basically associated with significant clinical and histologic inflammation (e.g., synovial inflammation) [40], and that is where NO comes into operation: Usually, the NF-kappaB activity is blocked by its association with the specific inhibitor IkappaB-alpha, resulting in a firm complex that prevents proinflammatory mediators (the effectors of NF-kappaB activation: TNF-alpha, IFN-beta, IL-1beta, IL-2, -6, -8) from being released [26]; cNOS-derived NO now stabilizes this IkappaB-alpha-NF-kappaB complex, thereby reducing the release of proinflammatory cytokines [26] - a process that is counteracted by iNOS-NO (see above; [26]). Thus, NO released via cNOS activity yet has profound antiinflammatory properties, not only by preventing the proinflammatory NF-kappaB effects but also by inhibiting NE- and CRH-related proinflammatory stress effects (see above; [41]). NO does not have antimicrobial properties alone but further mediates antiviral activity (e.g., IFN-gamma-related antiviral effects) [42].

With regard to tumor formation and its relation to NO, additional pathways have been detected. Interleukin 2-activated killer lymphocytes (LAK cells), induced after cancer formation or other activating immune processes, secrete inflammatory cytokines (such as IFN-gamma, TNF-alpha) that, in turn, induce NO synthesis [43]. This endogenous NO, even produced in susceptible cancer cells, may inhibit tumor growth (proliferation) and induce apoptosis [43]. However, high amounts of NO produced by activated

macrophages or external NO donors may be required to induce cytotoxicity and apoptosis in pathogens and tumor cells [44].

B. Infectious and parasitic diseases

Stress may play a significant role in infectious and parasitic diseases [13], as may NO. Table 1 gives information about NO functions and common pathophysiological patterns in selected infectious and parasitic diseases. In this context, AIDS (HIV infection) has not been listed in Table 1 but singled out and will be described more specifically, due to its complex nature and suitability to serve as an example.

Nitric oxide is detectable in the brain, and iNOS expression has been described in the virus-infected CNS [64]. Coat proteins of the HIV virus are able to influence NO levels - directly or with the support of IL-1beta -, a process that may lead to increased monocyte adhesion in blood vessels [64,65,66]. This mechanism may further facilitate the entrance of infected monocytes into body compartments (by enhancing transmembrane migration) [65]. Thus, although generally having various ameliorating immunological capacities, NO effects may predominantly be deleterious in HIV/ AIDS.

The addition of NO to HIV-1-infected blood mononuclear cultures produced a significant increase in virus replication, and virus replication was partially prevented by specific iNOS inhibitors [67]. Moreover, NO donors seem to enhance T cell infection [67]. These donors strongly boost HIV-1 replication in a dose-dependent manner, up to levels comparable to those achieved with TNF-alpha stimulation [67]. Further, iNOS inhibitors decreased virus replication in HIV-1 transfected T cells to similar levels obtained by administration of neutralizing anti-TNF-alpha antibodies [67]. Thus, while HIV-1 replication is also capable of inducing iNOS and TNF-alpha expression in T cells and T cell lines, NO appears to be involved in HIV-1 replication, especially in that facilitated by TNF-alpha [67,68]. Hence, NO (iNOS-NO) seems to play a detrimental role in HIV-1 infection [68], and iNOS expression may additionally be a key mechanism in severe AIDS dementia, neuronal injury, and neurodegeneration [64]. Furthermore, the high frequency of myocardial dysfunction found in HIV infection/ AIDS may also, in part, be due to - IL-1beta-associated - NO production [66]. Since IL-1beta stimulates NF-kappaB activity [66], the HIV-related enhancement of IL-1beta-induced NO production is also associated with activation of NF-kappaB, a process that may provide a previously unrecognized mechanism contributing to HIV cardiomyopathy [66].

Taken together, and in connection with other diseases (Table 1), two basic principles in infectious/ parasitic diseases obviously compete with each other: The need to fight an invasion or contain the infection on one hand, and the necessity to reduce inflammation and tissue damage on the other hand. NO and different cytokines are involved in both processes and have, in part, double functions. Thus, a fine balance may be required to coordinate and realize the illustrated, different goals.

C. Autoimmune disorders

Stress has been shown to play a major role in autoimmune disorders [13]. Again, NO pathways may be involved. In reference to pathophysiology, nitric oxide may be involved in positive (ameliorating) and negative (deteriorating) aspects of autoimmune diseases.

For example, iNOS-dependent tissue destruction and/ or disease progress have been seen in several rodent autoimmunity models, such as experimental allergic encephalitis (EAE, an experimental animal model of multiple sclerosis), uveitis (EAU), and glomerulonephritis [33]. One cascade for the development of organ-specific autoimmune diseases thereby invokes the induction and expansion of IL-12 and Th1 cells in response to microbial antigens, which then secrete IFN-gamma and that way activate macrophages and other effector cells for the production of tissue-damaging molecules (such as reactive oxygen intermediates and/ or NO) [33].

Thus, NO may be inductor and effector of cellular damage, and consequently, iNOS has been described to be generally associated with the pathogenesis of chronic inflammation [39]. However, NO release has also been demonstrated to limit autoreactive T cell determined spreading and diversification of the antibody repertoire, a process driven by macrophages [38]: NO may be important for silencing autoreactive T cells and may further restrict bystander autoimmune reactions following an innate immune response [38,69]. Here, the NO-related regulation of the Th1 cell response may represent a protective mechanisms against (negative) sequelae [33,37,70].

As illustrated above, the selective experimental inhibition of iNOS has been shown to exacerbate erosive joint disease [32,39]. Additionally, in the rat model of autoimmune interstitial nephritis, treatment with iNOS inhibitors has intensified the renal injury [71]. On the other hand, in EAU, genetic deletion of iNOS or low-dose treatment with a non-selective NOS inhibitor have been able to delay the onset and decrease the severity of the ocular inflammation, whereas a high-dose treatment with the same substance had previously been shown to actually exacerbate the disease/ symptoms [72]: NO (even derived from iNOS), besides mediating disease progress, certainly holds some protective functions in autoimmune diseases. These functions seem to be coordinated in balance and association with other NO effects. For further detailed information regarding underlying molecular mechanisms and the significance of NO in selected autoimmune diseases see Table 2.

D. Cancer/ neoplasms

Stress is speculated to be part of the cancer etiology [13,19,32,88,89]. This may be due to innumerable, diverse interactions, but the immune system is postulated to play a significant role (e.g., see [89]). Here, NO pathways are likely to be involved.

Nitric oxide is implicated in the control of malignancies. The expression of iNOS in tumor cells is generally associated with apoptosis, suppression of tumorigenicity, reduction of tumor growth, abrogation of metastasis, and even regression of already established cancer metastases [90,91]. Thus, NO has ameliorating capacities in cancer. In contrast, iNOS-NO may also promote tumor angiogenesis and metastasis [33,92]. Again, a complex system of biological pathways seems to exist and different NO strategies are interacting. For an appropriate functioning, a balanced tuning of the various mechanisms may be necessary. Thereby, the relevance of NO processes in vivo may differ from experimental findings. For example, high concentrations of NO tested in vitro may actually lead to nonspecific toxicity, in that way limiting the possible use of NO donors in the treatment of cancer [44].

The significance of specific (clinical) NO pathways has been demonstrated in a variety of malignancies. NO is prominently involved in skin cancer (melanoma) [91], brain tumors [29,92,93], breast tumors [29,44,92,94,95,96], lung tumors [92,97,98], pancreatic cancer [99], and colon tumors/ gastrointestinal cancer [29,43,100,101,102]. In hematologic malignancies, NO inhibits survival and growth of hematologic cancer cells (via TNF-associated pathways) [103]. Thus, NO - as part of auxiliary autoregulatory 'efforts' - exhibits antileukemic and apoptosis-mediating activities [103]. However, in B cell chronic lymphocytic leukemia, iNOS actually seems to facilitate the malignant cells' resistance to the normal apoptotic path [104]. This anti-apoptotic role of NO may be due to an inhibition of caspase activity [104]. In contrast, the apoptotic action of NO found in breast cancer cell lines is induced via cytochrome c and caspase-9, -3 activation [44]. Here, a better knowledge of the mechanisms governing the ultimate effect of NO, anti-versus pro-apoptotic, is still missing. Yet, this knowledge would potentially allow the development of new therapeutic approaches for the treatment of malignant diseases [104].

In brain, breast, lung, and colon tumors, a complex picture for the activity of NO occurs: A high-output NO production by infiltrating macrophages, for example, can induce cytostasis and/ or cytotoxicity, whereas a low-output NO production within the tumor may increase tumor blood flow and promote angiogenesis [33,92]. Moreover, NO produced by the tumor itself may inhibit proliferation or induce apoptosis of T lymphocytes, which could explain the suppression of host immune functions often observed to go along with tumor growth [32]. In this context, tumor-promoting effects of NO in lung tumors have been considered to be due to an inhibitory effect of (iNOS-) NO on the 'host' immune response, accompanied by a NO-associated enhanced vascular permeability (and angiogenesis) [98]. A similar combination of potentially adverse NO pathways has also been detected in colon cancer, yet NO synthesis has further been demonstrated to be linked to IL-2-activated killer lymphocyte (LAK cells) activation, a cytotoxic mechanism that induces growth inhibition and programmed cell death in susceptible cancer cells [43]. Thus, the role of iNOS/ NO in cancer is complex and appears to be paradoxical: NO may trigger cancer initiation or progression on one hand and protection or regression on the other (in part, by using the same receptors) [98]. Again, the timing of NO activity, actual NO levels, and the balance between different NO pathways are crucial factors for the promotion of either beneficial or detrimental NO effects.

E. Others

For some diseases with great clinical importance whose definitive etiology still remains unclear and/ or whose placing within the immunological domain is controversially discussed, stress and NO pathways are often considered relevant. Thus, atopic dermatitis, psoriasis, celiac disease, and ulcerative colitis/ Crohn's disease are all presenting a close association with stress [13,36,41,105-109], and NO, parallel to situations already described, plays a significant role in coupled protective or ameliorating processes [13,41,110,111]. However, as seen before, NO pathways may also turn out to be detrimental in sporadic or specific situations, and here, most often iNOS and related proinflammatory cytokine-driven processes are of importance [102,111-118].

E. Cardiovascular diseases

1. Hypertension

Stress has been shown to be important in vascular hypertension [6]. It may serve as a risk factor [119], induce blood pressure spikes, or increase an already elevated blood pressure [19,36,120]. Stress may even, in part, cause or contribute to the clinical onset of arterial hypertension in certain cases [121,122,123].

Etiology and pathophysiology of hypertension are complex. Besides primary, essential, or idiopathic forms, symptomatic (secondary) forms exist. Aging, atherosclerosis, other risk factors, and sympathetic nervous system activity (→stress) may all play critical roles [124]. Further, nitric oxide pathways are involved: Non-selective NOS-inhibition has been demonstrated to induce hypertension [33,125]. On the other hand, prolonged times of increased blood pressure may in turn lead to a decrease in vascular pulsations and thereby reduce NO levels [126]. Yet, basal (constitutive) NO seems to be partly regulated via arterial vascular pressure pulses, and eNOS, for example, has been shown to be important for the regulation of basal blood pressure [126]. Thus, NO may function as an ambivalent signaling molecule promoting complex autoregulatory pathways involved in blood pressure regulation.

Looking at its beneficial properties, NO may be considered a possible therapeutic agent in hypertension: Since NO inhibits NE-dependent vascular contraction and is capable of lowering arterial blood pressure, vascular responsiveness to contractile substances like NE may be significantly attenuated by prior or subsequent exposure to NO [23,125]. Additionally, an impaired NO synthesis (in mice) has been demonstrated to result in increased sensitivity to the pressor effect of mineralocorticoids in the presence or absence of an increased saline intake [127]. Therefore, NO may actually decrease the blood pressure in mineralocorticoid-sensitive cases [127]. This possible contribution of NO pathways to the adaptive response to mineralocorticoid excess may point out an impact of NO on natriuresis [127].

Although little is known about potentially deleterious effects of NO in hypertension, the long-term therapeutic use of NO in the form of externally administered drugs must be considered carefully: over time, autoregulatory pathways will become activated in response to regain a dynamic balance and may introduce deteriorating mechanisms or focus on less suitable physiological setpoints. Endogenous therapeutic tools, in contrast, may possibly be more appropriate - if available.

2. Atherosclerosis, endothelial dysfunction

The pathophysiology of atherosclerosis seems to be complex and many etiological factors may be of importance. Clearly, stress has the capability of representing or becoming a crucial factor in certain cases of atherosclerosis-related disease processes [6]. Further, high-fat diets (e.g., high-cholesterol, saturated fats) can induce atherosclerosis [128,129], and atherosclerosis caused by moderate hyperlipoproteinemia is highly susceptible to the influence of psychosocial stress [130]. Since oxidative stress may induce endothelial dysfunction and injury, and since endothelial injury has been considered an initiating

event in atherogenesis [128], oxidative stress/ free radical activity may also contribute to the pathophysiology of atherosclerosis [131].

Nitric oxide plays a major role in endothelial dysfunction and atherosclerosis. NO pathways appear to be predominantly protective, but, since the free radical enhances oxidative stress, NO can also exert deteriorating effects. NO usually induces a vasodilation, whereas endothelin, its physiological counterpart on vascular levels, typically induces a strong vasoconstriction [132]. Both are synthesized in endothelial cells to maintain a natural balance between the different effectors of the vasomotor regulation [132]. When this balance gets substantially unsettled, endothelial dysfunction may occur [29,32,133,134]. Thus, atherosclerosis may be caused by an impairment of endothelial cell-related NO synthesis and NO-dependent vasodilation (i.e., endothelial dysfunction) [29,133,134].

Native (LDL-) cholesterol is harmless, but when oxidized (e.g., via oxidative stress) it becomes deleterious: it 'consumes' eNOS-NO, thereby reducing the vessel's ability to induce vasodilation when needed, thus giving way to endothelial dysfunction and atherosclerosis [132,135]. In contrast, an increase of NO bioavailability may result in the regression of preexisting atherosclerotic lesions: eNOS-NO has an antiatherogen potential [136]. This description may be specifically important for patients with hypercholesterolemia [135]. Oxidized LDL is cytotoxic and chemotactic: it attracts macrophages that 'digest' the vascular LDL-deposits, get 'fatty', eventually die and/ or build foam cells, fatty streaks, thereby inducing plaque and, in consequence, atherosclerosis [132,135]. Hence, atherosclerosis can also be interpreted as a local inflammation, caused - among others - by oxidative stress, enhanced (LDL-) cholesterol, endothelial dysfunction, insufficient constitutive NO mobilization, and activated (pro)inflammatory pathways.

Positive effects of constitutive NO on reducing proinflammatory cytokine synthesis may contribute, in part, to its antiatherogenic and antiinflammatory properties [26]. Additionally, NO is known to be capable of inducing apoptosis, and apoptosis of vascular smooth muscle cells may represent a critical step in therapeutically counteracting atherosclerosis (as it may even prevent intimal hyperplasia in restenosis) [26,137]. However, the particular signaling pathways still remain unclear [137]: The experimental induction of apoptosis in human (coronary artery) smooth muscle cells by exogenous NO has been demonstrated to involve protein kinase C signaling and the regulation of NF-kappaB binding activity [137]. Further, an experimental NOS inhibition enhances leukocyte rolling flux, adhesion, and vascular emigration (in atherosclerosis-prone areas) in an angiotensin II-dependent manner [125]. Thus, leukocyte recruitment apparently culminates in vascular lesions that occur in hypertension, atherosclerosis, and myocardial ischemia-reperfusion injury [125]. Constitutive NO may serve as a protective agent here. But still, many aspects of this molecular 'picture' are unknown.

The detrimental aspects of pathophysiological NO pathways also become relevant in atherosclerosis: iNOS has been found in atherosclerosis-coupled macrophages, foam cells, and vascular smooth muscle cells [29,32]. The associated cytokine profile includes IL-1, -6, -12, TNF-alpha, and IFN-gamma [29,32,133,134]. Thus, an imbalance between constitutive and inducible NO pathways (in support of the latter) may lead to enhanced immunocyte attraction, inflammation, endothelial damage and dysfunction [26].

3. Coronary artery disease

Coronary artery disease (CAD) is the number one cause of adult mortality due to a medical illness in the United States [138]. In CAD, the main concern is the possible myocardial ischemia that goes along with CAD, i.e., chronic or acute ischemic events [138]. In general, coronary artery disease describes a special form of atherosclerosis that manifests itself in the coronary arteries. Thus, both fields overlap and what has been demonstrated for atherosclerosis may almost be transferred and adopted here. For example, as with atherosclerosis, CAD is strongly associated with stress [6,18,139,140]. However, we won't repeat the basic analogies between atherosclerosis and CAD here. Instead, we will focus upon some specific or particularly pronounced pathophysiological aspects of CAD - especially those that are related to stress and/ or nitric oxide pathways.

Many studies regarding the pathophysiology of CAD have been conducted over the past decade - an indicator for the importance of this disease, related to its high mortality/ morbidity in industrialized countries [138]. Hence, a great number of risk factors for CAD have been detected so far, such as: Inactivity, hypercholesterolemia, hyperhomocysteinemia, hypertension, diabetes mellitus, smoking, obesity, aging, and endothelial dysfunction/ NO-imbalance [122,132,135,136,141-144]. Most of these conditions are associated with increased oxidative stress, particularly with increased production of superoxide radicals and elevated levels of oxidized (LDL-) cholesterol, and both factors can attenuate the biological activity of the protective eNOS [122,132,135,136]. Thereby, superoxide anions and oxidized LDL have repeatedly been shown to cause or exacerbate CAD, and these factors can be decreased by administering antioxidants like vitamin E and C (moreover, vitamin C also stabilizes eNOS-NO) [122,135,136]. Thus, nitric oxide is involved in various (patho)physiological processes associated with CAD [138]. Furthermore, NO plays a major role in endothelial function, and this fact may be important for certain myocardial perfusion abnormalities as well [122,135,136]. Taken together, NO appears in CAD, endothelial (dys)function, and related problems on different levels, primarily via its constitutive forms and in a protective, ameliorating context. However, deteriorating NO pathways are also imaginable - since they are also part of underlying mechanisms involved in endothelial dysfunction (via iNOS), but little is known to this point about NO and its detrimental capacities in CAD.

Studies have found that the coronary microcirculation of patients with atherosclerosis may be dysfunctional: Patients with CAD do not show the normal microvascular dilation/ endothelial function during mental stress [145], a response (likely mediated by alpha-adrenoceptor activation) that may contribute to myocardial ischemia [145]. Further, a chronic sympathoadrenal activation - together with behavioral, environmental factors - may lead to CAD [130,146], and a therapeutic beta-blockade may prevent this type of CAD [146]. Finally, the triad of hypercortisolism, ovarian impairment, and psychiatric morbidity (as seen in 'stressed' premenopausal female monkeys) may represent a high-risk state for disorders of the cardiovascular system [147], thereby indicating the multifactorial nature of CAD pathophysiology: Stressors/ stimuli, exogenous and endogenous conditions (including genetic predispositions), imbalances between the different factors, and diverse molecular pathways - including NO signaling pathways - are of importance.

4. Myocardial infarction

Myocardial infarction (MI) describes an ischemic event that follows an acute interruption of a sufficient coronary blood supply, usually going along with CAD, coronary spasm, thromboembolism, arrhythmia, trauma etc. [148,149]. Beyond doubt, stress has the potential to actively trigger this threatening cardiac event [6,150,151,152], and here, mental stress appears to be exceptionally potent [145,152,153,154].

The pathophysiological events surrounding myocardial infarction resemble those involved in inflammatory responses described earlier. Again, nitric oxide plays a significant role, and a functional distinction between iNOS- and cNOS activity may also be detectable: NO seems to have conflicting functional properties and, besides deleterious effects, ameliorating aspects of NO have yet been detected in MI as well. With regard to the latter, NO may actually provide protection against post-ischemic tissue injury by preventing adhesion of polymorphonuclear leukocytes (a mechanism that involves constitutive NO pathways) [26], and further, NO may decrease chances of myocardial ischemia/ reperfusion injury by preventing the hyperdynamic (contractile) response during early reperfusion [155]. Moreover, ischemic preconditioning, involving NO pathways, may protect against detrimental consequences of myocardial ischemia [138].

However, the administration of iNOS inhibitors in an animal infarction model has been shown to improve ventricular performance and increase myocardial blood flow in the surviving myocardium - suggesting potentially detrimental, tissue-damaging effects of iNOS in MI [29,156,157,158,159]. Additionally, the human iNOS promoter contains a hypoxia-responsive element, and iNOS has been detected in cardiac myocytes and infiltrating macrophages several days after MI [29,158,159]. Also, iNOS expression, seen in connection with MI, seems to correlate with contractile dysfunction, apoptotic cell death (of cardiac myocytes), and local inflammation [157,158,160,161,162]. During myocardial ischemia/ reperfusion, NF-kappaB is activated, a process - likely triggered by iNOS induction - that leads to the up-regulation or expression of inflammatory cytokines (e.g., IL-1, -6, TNF-alpha) [26,118,162]. Thus, iNOS apparently has detrimental capacities in MI, and NO may even serve as a mediator involved in the myocardial ischemia/ reperfusion injury [163]. Again, a balanced state of adequate NO activity and a coordination of well-adjusted (various) NO pathways may therefore be an important 'goal' in MI (also: see [138]).

5. Others

In non-insulin-dependent diabetes mellitus (NIDDM), a causal association with stress (that is, with an 'excessive stress response', CRH-hyperactivity) has been discussed recently [6,164]. Moreover, a lack of tonal (constitutive) NO levels may be of pathophysiological importance [26,118].

Stroke has been associated with psychosocial stress [6,165]. As seen before, NO released via (constitutive) eNOS pathways seems to possess protective capacities [26]. In contrast, iNOS activity may exert detrimental effects, and a long-lasting/ high-output iNOS-NO production - eventually observed in stroke - may even become strongly neurotoxic [26,29,166].

Cardiomyopathy (CM) has been said to be associated with stress in particular cases [6,167]. Nevertheless, the concrete connection remains still unclear and underlying mechanisms are not well understood yet. However, iNOS expression and related NO pathways have been found in dilated and ischemic cardiomyopathy, and iNOS (proinflammatory) activities may be associated with genesis and pathophysiological processes involved in CM [66,118,156,157,168].

In the aging cardiovascular system, an increased overflow of NE to the plasma frequently occurs, presumably due to a reduced NE reuptake following sympathetic stimulation [6,169]. This effect is particularly observable after administering or experiencing mental stress [169]. Thus, an 'excess' of NE may regularly appear in older people [122,169]. Further, aging increases human sympathetic nervous system (SNS) activity at rest [169], thereby potentially enhancing frequency and intensity of possible stress responses [169]. However, this fact may be modified by a parallel decreased SNS reactivity - which may, in turn, reduce an elevated risk related to sympathetic (hyper)arousal [169,170]. Nevertheless, oxidative stress is increased in older organisms (NO pathways may be involved), and this may contribute the pathophysiology of atherosclerosis and other chronic diseases prevalent with aging [131].

Stress has been identified as a contributor to chronic heart failure (CHF) [6]. Here, sympathetic (hyper)arousal may be involved [171]. Thereby, neuropeptide Y coexists with NE in sympathetic nerves and is co-released on sympathetic activation: Cardiac failure is associated with an increased release of NE and neuropeptide Y from the resting - and the stimulated/ 'stressed' - heart [171]. Further, endothelial dysfunction is a key feature of CHF, contributing to enhanced peripheral vasoconstriction and impaired exercise capacity (exercise intolerance) [172,173]. Hence, regular physical exercise increases constitutive eNOS-NO and (endothelium-dependent) vasodilation of the skeletal muscle vasculature in patients with CHF: The correction of endothelial dysfunction via exercise may be mediated by constitutive NO, and eNOS-NO release may therefore be associated with an improvement of exercise capacity [172]. In contrast, the expression of iNOS may display a deleterious relationship between CHF and endothelial dysfunction: iNOS, known to be part of the pathophysiology associated with endothelial dysfunction, is expressed in CHF [156,168,173,174], and inducible NO-derived pathways may thus be crucial for the development of CHF [156,157]. Additionally, apoptosis in skeletal muscles and cardiac myocytes of CHF patients appears to be enhanced, leading to reduced work capacity and contractile force detected in these patients [168,174]. Again, iNOS pathways seem to participate [168].

6. Neurodegenerative diseases, mental disorders

In neurodegenerative diseases and mental disorders, stress clearly plays a significant role [7]. Moreover, similar combinations of detrimental influences of stress and NO-related (patho)physiological pathways may exist (see above). Thereby, potentially underlying mechanisms and involved interconnections may resemble those found in other diseases (e.g., immunological or cardiovascular diseases). For example, multiple sclerosis (MS) shows pathophysiological connections with experimental autoimmune/ allergic encephalitis (encephalomyelitis, EAE) [105], but the definite etiological classification of MS still remains unclear. However, EAE and MS demonstrate a possible association with

stress [13,18,105,175], and iNOS expression has also been detected [29,176]. Thus, MS - interpreted as a (pro)inflammatory disease - apparently involves NO-driven cytokine activation (identified cytokine profile: IL-1,-2,-6, TNF-alpha, IFN-gamma), but protective NO capacities have also been described [13,29,118,177,178]. Comparable findings have further been obtained in Parkinson's disease [126,179]. The similarities (regarding underlying mechanisms) found in neurodegenerative and other diseases will now be exemplified in a particular neurodegenerative disease in the following, namely one with growing clinical significance: Alzheimer's disease.

7. Alzheimer's disease

Stress has been shown to be involved in neurodegeneration (overview: see [7]). Furthermore, stress has been demonstrated to cause deficits especially in spatial memory performance [180,181,182,183], and this effect may be important for pathophysiological processes connected with Alzheimer's disease (AD). Additionally, a hippocampal atrophy may be involved [7,181,182], and the stress-related activation of the SNS and/ or HPA axis may represent a pathophysiological 'starting point' here [7,182]. Thus, stress may lead to a loss of neurons, particularly in the hippocampal area [7,182]. Moreover, the aging hippocampus apparently is more susceptible to stress, and this vulnerability may yet be increased in AD [184].

Strenuous exercise, taken to the extreme, initiates an immune and vascular proinflammatory response (i.e., 'excessive stress response'), whereas mild exercise seems to produce more health benefits [126]. Hence, the mentioned proinflammatory response may exert detrimental effects on neuronal integrity. Differences in (patho)physiology between strenuous and mild exercise may indicate distinct forms of NO production (iNOS versus cNOS-derived NO release) [126].

In light of negative effects of stress on memory performance and neuronal integrity (coupled to NO pathways), nitric oxide may be part of underlying mechanisms involved in AD. Recent studies have shown that impairments in hippocampal-dependent memory consolidation produced by agents that are also capable of inducing IL-1beta activity are blocked by antagonizing IL-1beta (e.g., via cNOS-NO) [7,26,180]. Additionally, NO appears to modulate learning and memory: Decreased levels of NO coincide with reduced spatial learning capacities [26]. Therefore, constitutive NO may be beneficial in AD. Further, mild exercise may be helpful in AD via mimicking pulsatile blood flow, since cyclic pulsations, found in exercise, apparently increase (constitutive) NO [126] - whereas a reduction of vascular pulsations (e.g., observable in prolonged times of increased blood pressure) leads to decreased NO levels [126]. However, iNOS expression has also been detected in the CNS (at critical points): Glial cells/ astrocytes and neurofibrillary tangle-bearing neurons (AD) show iNOS activity [185,186]. Thus, NO may also be part of AD's pathophysiology.

In AD, the actual significance of findings connecting neurons with a high susceptibility to the cytotoxic action of (inducible) NO is not yet clear. Nevertheless, excessive NO levels are responsible for certain kinds of neurotoxicity in the CNS, even detectable in AD [26,64]. Thereby, NO appears to either have a neuroprotective or neurotoxic function - depending on its concentration and the redox state of the tissue [186]. Both, iNOS and cNOS, may actually have similar properties here, distinguishable only by different levels

and timing of NO release (different patterns of activity) [26]: iNOS and cNOS may potentially exert a down- or up-regulation of oxidative stress - according to given circumstances or the specific state of regulation/ balance [26]. In this context, elevated levels of oxidative stress (e.g., via increased NO concentrations) may represent a pathogenic factor in AD [187,188].

NO production by microglial cells, astrocytes, and brain microvessels is enhanced in patients with AD [188]. Further - although (pre)beta-amyloid processing apparently is the most crucial step in AD pathophysiology [7,186] - sporadic AD may also develop from cerebral capillary endotheliopathy [189]. Thereby, advanced aging and vascular risk factors may lead to a 'critically attained threshold' of cerebral hypoperfusion (CATCH), a hemodynamic microcirculatory insufficiency that can destabilize neurons, synapses, neurotransmission, and cognitive function, creating in its wake a neurodegenerative state characterized by the formation of senile plaques, neurofibrillary tangles, and amyloid angiopathy (and in some cases Lewy bodies) [189]. It has been proposed that CATCH may initiate AD by distorting regional brain capillary structure (progressive brain capillary degeneration), involving endothelial cell (EC) shape changes and impairment of endothelial NO release, which then may affect signaling between the immune, cardiovascular, and nervous system [189]. Here, lower eNOS-NO levels may lead to prolonged cellular excitatory states and EC size or histological changes - eventually causing hemodynamic disturbances, mitochondrial dysfunction, cellular death/ neuronal 'malnutrition', and neurodegeneration [189]. Thus, AD may be interpreted, at least in certain cases, as a microvascular disorder that is associated with NO pathways and aging [189]. However, these mechanisms would not explain primarily familial or early-onset AD.

AD involves neurodegenerative and inflammatory processes: Neuronal decrease, connectivity loss, and glial reactivity have been detected in AD [187], and the main processes implied in the neuronal cell death are presumably started by different factors - such as a lack of neurotropic agents, (chronic) hypoxia/ hypoglycemia, excitotoxicity, and oxygen/ nitrogen free radicals [187]. Additionally, a DNA polymorphism at the angiotensin converting enzyme (ACE) gene has also been linked to the risk of late-onset AD [188], and the plasticity and resilience of brain cells to stress hormone action (especially corticosteroids) may be altered in AD [184]. After all, imbalances between protective and detrimental, neurodegenerative or inflammatory, factors may play a highly critical role in AD's pathophysiology.

8. Anxiety, depression

The possible association of anxiety and depression with stress has been discussed recently [7]. Thereby, stress - in general - has been demonstrated to be part of mechanisms related to anxiety [164], and chronic stress, involving chronic sympathetic activation, has specifically been linked to the onset of anxiety and depression [190].

A few underlying molecular mechanisms with pathophysiological significance have been suggested in anxiety/ depression thus far. A pathologic hyperactivity of the stress response system ('excessive stress response') has been discussed in association with anxiety disorders, and apparently, this type of stress response is often a product of an experienced trauma in childhood/ youth [164]. In contrast, a 'secure environment' seems

to protect against stress-related illnesses [164]. Further, CRH enhances the organism's sensitivity to noxious stimuli and may be capable of mobilizing nearly the entire cascade of the stress response [164]: A hyperactivity of CRH (facilitating enhanced cortisol levels) may be, in part, at the bottom of depression and anxiety [164]. Hence, there appears to exist a significant correlation between mother's extent of depressive symptomatology and her child's cortisol levels, and additionally, children with low socioeconomic status present a significantly higher salivary cortisol level than children with high socioeconomic status [191].

However, the concrete relationship between an excessive activation of the HPA axis, its triggers and variables (including cortisol levels), and clinical depression is still a matter of discussion. Nevertheless, stress response pathways ('excessive' or 'inadequate'), serotonin-deficiency, and hypercortisolism are among the most likely factors to promote the multi-component pathophysiology associated with depression and anxiety [19,105,164]. Here, even melatonin may play an important role, since decreased melatonin levels represent an accepted key feature of 'winter depression' (seasonal affective disorder) [192].

Nitric oxide may also be involved in the (patho)physiology of anxiety/ depression, because it interferes with various components and underlying mechanisms of the stress response on different levels, thereby potentially exerting protective or - simultaneously - detrimental effects (described above). In an animal (rat) model for chronic mild stress - mimicking human depression -, the detectable dendrite atrophy/ deformation of neurons in the hippocampal formation (experimentally related to an overproduction of NO) was inhibited by fluoxetine, an antidepressant that is capable of blocking NO production [193]. Thus, the reduction of a possibly exaggerated NO pool in depressive patients via fluoxetine may allow a reorganization and renormalization of deformed hippocampal neurons [193]. In addition, other reports have addressed the involvement of NO in depression [194]. For example, NO apparently is associated with various depressive symptoms, such as sexual dysfunction, weight loss, psychomotor retardation, indecisiveness, and irritability [194]. However, the connection between NO pathways and symptoms of depression does not necessarily imply a causal pathophysiological association between NO and depressive disorder. Further research is needed to verify possible implications. Yet, the stress (patho)physiology with its two main components (SNS and HPA axis), glucocorticoid and CRH pathways, hippocampal deformation/ neurodegeneration, and NO signaling are closely interconnected (see above), and mental disorders like anxiety and depression represent an area where these components/ mechanisms regularly come into play [5,6,7,193]. Thus, a relevant pathophysiological association between NO and depression, for example, is certainly a possibility.

Discussion

Many stress-related diseases exist, and the field of stress in connection with its impact on health still appears to grow, especially in the 'western world' [1,6,7,13,195]. In fact, stress is a major contributor to most of the diseases and complaints seen in primary care practices [1,4].

Not all stress appears to be dilapidating. Following a stressor, survival and balance usually are maintained within a steady, well-tuned range by activating various adaptive

autoregulatory cascades. These mechanisms, eventually leading to the desired ('re-balanced' or 'adapted') conditions, involve biological, psychological, and sociological corrective measures [22]. Thus, stress responses can exert protective effects - but for executing this, underlying mechanisms and autoregulatory cascades must be instantaneously accessible in a stressful situation, and therefore, their effectors must be expressed constitutively [22]. NO meets this criteria: NO is constitutively released via its cNOS pathways, and this NO, due to its lower levels and shorter boosts following stimulation (compared to inducible NO pathways), may predominantly exert protective or ameliorating functions. Hence, one of the actions of NO may be to directly counteract the effects of the stress response and its effector NE, thereby decreasing sympathetic activity (see above).

NO has already been implicated in a great number of physiological functions (such as NE and dopamine release, memory and learning, regulation of cardiovascular and immune systems, regulation of stress response pathways, stabilization of neurons, modulation of wakefulness, modulation of nociception, olfaction, food intake, and drinking), but still, many more areas of importance with regard to NO involvement are under investigation [6,7,13,21,194]. However, NO has also been related to various pathologies (see above). Moreover, stress and a related secretion of NE apparently cause or exacerbate many different disease processes [6,7,13]. Again, NO may play a significant role. Thus, NO and stress certainly have detrimental capacities, and these seem to be predominantly associated with higher NO levels and longer periods of elevated NO concentrations, both conditions that may be obtained by iNOS expression (described above). This inducible form of NO production may therefore represent an important part of the potentially hazardous stress response pathways.

Stressful stimuli that lead to the secretion of stress-related NE (and glucocorticoids, NO) may impede our evolutionarily developed natural healing capacities. Nevertheless, activation of the critical stress response components (e.g., SNS and HPA axis) still represents a primarily adaptive mechanism in appropriate situations - like acute disease processes and biological challenges. Yet, in more severe or chronic states of diseases, a more rigid and non-flexible regimen may take over. Again, NO may be involved, but here, the detrimental effects of (inducible) NO may play a more significant role than the ameliorating capacities. Additionally, in more chronic situations, an organism may become more vulnerable or susceptible to negative aspects of stress response pathways, and a state of balance may be 'out of sight' - whereas in less rigid, less severe, earlier disease states, flexibility may still be possible and NO may eventually be helpful [22]. Thus, the key step for normally (and otherwise) useful physiological mechanisms becoming pathophysiological - i.e., leading to more serious disease states - may be represented by the loss of balance, the loss of control over the different pathways induced. In addition, our physiological or psychological stress response systems presumably have been designed to function for short not prolonged periods of time. With regard to the latter, the failure to terminate or shift actually protective mechanisms (that now have started to do harm, e.g., via facilitating excessive NE, glucocorticoid, or NO levels and/ or iNOS expression) towards a state of 'healthy balance' may lead to a vicious cycle of disease-supporting pathophysiological pathways.

Common underlying molecular mechanisms exist that represent a connection between the stress response and pathophysiological findings in stress-related diseases (particularly with reference to NO). Again, NO holds ambivalent capacities: Small quantities produced by cNOS may mediate physiological - protective or ameliorating - effects, whereas iNOS expression (or an overstimulation of cNOS) may lead to large quantities of NO, a situation that may be associated with cytotoxic or negative NO effects detectable in various disorders [31]. Thus, NO activity and pathways have to be coordinated and balanced [138]: While acute stress can induce protective mechanisms [6,7,13,138] - and these can become activated in different systems (e.g., immune [13], cardiovascular [6], or nervous system [7]) -, the underlying physiology often seems to be associated with well-balanced constitutive NO pathways [138]. However, chronic or overwhelming stress (or a disadvantageous predisposition) may put this balance at risk or even destroy it. As a result, this perturbation of cellular homeostasis may, for example, trigger NF-kappaB activation that results in proinflammatory DNA promoter regions becoming activated [13,118,138]. Although having distinct positive and beneficial capacities as well, proinflammation may actually underlie a variety of severe pathophysiological disease processes, possibly as a common (pre)condition that only manifests itself differently (leading to different affected regions or different diseases) [13,118]. Therefore, common pathophysiological analogies exist between different stress-related diseases, and these significant similarities/ underlying mechanisms are associated with NO pathways (at least in part). After all, the described NO-related pathology (i.e., stress pathophysiology) may represent an interesting field for future therapeutic strategies, including stress management and relaxation response techniques.

Conclusions

Profound connections between stress and various disease processes exist. Thereby, common pathophysiological pathways in stress-related diseases have been described, and they involve stress hormone (cortisol, norepinephrine) and, in particular, nitric oxide activity. Thus, NO has detrimental capacities. However, NO not only exerts deleterious but also strongly ameliorating effects. The balance between both properties is crucial. Dynamic biological balances are generally relevant in stress-related processes. Nonetheless, the precise conditions under which a healthy balance between disease-promoting and -ameliorating factors may occur still have to be defined for most stress-associated disease processes. Yet, nitric oxide involvement in stress-related diseases represents a common pathway, with various pathophysiological analogies, that may be accessible for strategies using stress management and relaxation response techniques.

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