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Review

The placebo effect and relaxation response: neural processes and their coupling to constitutive nitric oxide

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Abstract

The placebo effect appears to be a real phenomenon as is the scientifically demonstrated and examined relaxation response. Given this, we attempt to understand how these phenomena work in light of our current understanding of central and peripheral nervous system mechanisms. Central to our hypothesis is the significance of norepinephrine, nitric oxide and opioid signaling both in the central and peripheral nervous system. In this regard, we find that nitric oxide controls norepinephrine processes on many levels, including synthesis, release and actions. In closing, we conclude that enough scientific information exists to support these phenomena as actual physical processes that can be harnessed to provide better patient care.

Theme: Neural basis of behavior

Topic: Motivation and emotion

Keywords: Placebo; Relaxation response; Nitric oxide; Belief; Limbic system; Norepinephrine

Contents

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1. Introduction

It is reasonable to propose that our bodies contain naturally occurring antibiosenescent processes, including immune, vascular and neural systems, that serve to maintain our health for a reasonable period of time, and that these processes in part determine our mean life span. In most mammals, once these protective systems diminish in their capacity, their reproductive life has also ended. In man, however, in part aided by our integrative capacity, our life span is extended well beyond our reproductive years. It would not be surprising, therefore, to find critical neuronal processes linked to man's cognitive ability that have the ability to promote health. These processes would manifest themselves during times of stress, when an increase in a health-related cognitive stimulus initiates this innate, non-cognitive protective neural process to become evident. We speculate that the above hypothesis in part explains these two phenomena: the relaxation response and the placebo effect. With this focus, we will attempt to explain how this cognitive awareness of health issues may activate these non-cognitive, protective neural processes.

2. What is a placebo?

The word 'placebo' means 'I shall please' in Latin, and is the first word of the church vespers sung for those who have died [19]. In 12th century Europe, the word 'placebo' was shorthand for these vespers. By the 1300s the term had been adapted in the secular vernacular to mean 'false consolation' since insincere mourners were paid to sing these placebos. When the term entered the medical lexicon in more modern times, it preserved this negative connotation as something inactive, given not to sincerely aid a patient but rather to please them temporarily. Before Koch and Pasteur's germ theory of disease, the history of medicine was largely the history of the placebo effect [8], but physicians after the mid 1800s increasingly disregarded the significance of placebos. Physicians no longer viewed the placebo effect as an ally in the fight against disease, as modern science could not establish a link between placebo and changes in disease processes. Recently, the shift towards patient autonomy and the transformation of our society into one based mainly on science has led us to taboo the notion of belief in medicine [107].

Nevertheless, Beecher in 1955 reported a 35% average placebo response rate in conditions such as pain, coryza, high blood pressure, headache, seasickness and drug-related mood disturbances, and Benson in 1979 reviewed studies claiming even higher rates of effectiveness in angina pectoris, asthma, herpes simplex and duodenal ulcers [5,12]. Modern medicine is indeed at its zenith as a healing profession because of its scientific advances in surgical and pharmacological therapies. Healing can be enhanced, however, by the power of the placebo 'to please', it seems, by tapping into the positive expectations and beliefs of the patient [19,107]. How does this positive mind state or 'remembered wellness' as Benson calls it, affect a healthy physiological profile?

In experimental clinical trials the placebo represents a lack of treatment, used in a control capacity so as to better evaluate the effects of the 'active' drug in question. In double-blind trials, neither the patient nor the examiner knows which compound is the real substance and which is the placebo. Counter-intuitively, however, something interesting has emerged from this type of scientifically controlled study. Namely, those individuals given the placebo often exhibit statistically significant improvements, suggesting that treatment with the placebo itself has a beneficial effect. The placebo effect may really represent the manifestation of a proactive mind–body link that evokes an innate protective response (Fig. 1). The cognitive stimulus that elicits this non-cognitive protective response to a perceived health threat may be the patient's belief in:

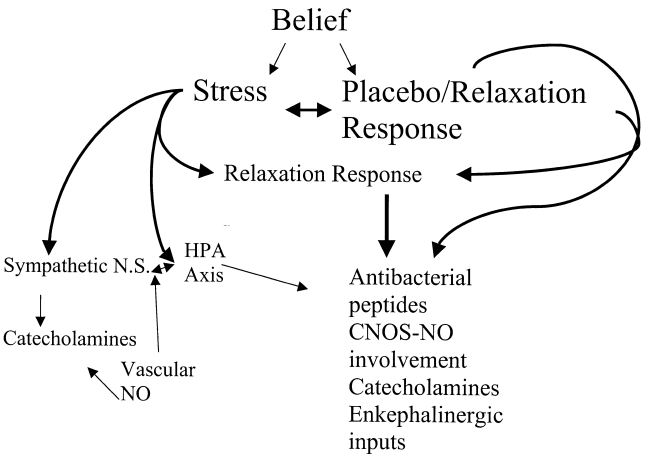

Fig. 1. Illustration of the interrelationship of stress, belief and how they may impact on the placebo effect and the relaxation response. Beliefs can clearly influence what is perceived as a stress. In this regard, both can impact on a physical plan of action, i.e., placebo effect. Thus, components of the placebo effect and its physical learned component, relaxation response, can encompass aspects of the stress response, i.e., norepinephrine involvement. Thus, counter-intuitively, the relaxation response can and does stimulate the sympathetic nervous system. In regard to 'pure' stress that's allowed to continue, this 'stress' pathway leads to hypothalamic–pituitary adrenal axis (HPA) activation as is classically referred to as the stress response. Indeed, as recently demonstrated this pathway can receive input from the peripheral vasculature via endothelial nitric oxide initiating neurosecretion [17,90,92,93]. Furthermore, motor responses by way of both the placebo/relaxation response and the HPA axis may also activate various constitutive processes as noted. The key phenomenon is that during the placebo effect and the relaxation response the HPA axis activity, including a prolonged norepinephrine action, is diminished. Thus, the constitutive processes are 'turned on' during our response to stress and the placebo/relaxation response, but if belief and learning can impact on these processes and they are not organically damaged, this can diminish the stress response, allowing for the constitutive processes noted above to predominate. The end result will be the enhancement of general health. This is why the relaxation response has been shown to impact on so many pathological conditions (see text).

the doctor, the 'medicine', the treatment, themselves and their value system, God and/or a combination of these factors, including the relationship of the doctor and patient. This may be unique to humans, since as far as we know other mammals do not exhibit the placebo effect, though they may access health benefits from the social comfort they clearly enjoy. The exact processes involved in this protective response are unknown.

With regard to human history, we have always been aware of this innate protective response. For example, in Greek culture, 2000 years ago, it was believed that the healthy mind and the healthy body went hand-in-hand, establishing a link between mind–body experiences. This type of association can be found in many societies/cultures throughout human history, and it even exists in present day society. Anecdotally, we have always perceived that there may be more to health maintenance than just a particular pathologic situation, suggesting that the mind's cognitive as well as non-cognitive link to the body may be involved in a proactive manner with promoting our health. Additionally, given the evolution of cognition, we propose that we may be able to initiate this innate proactive health-oriented process at will. Furthermore, the existence of such a protective process can be surmised from human longevity; mechanisms must exist to promote our health for this extended period of time (Fig. 1). In this regard, the mind–body link can be viewed as an antibiosenescent process.

3. What is the relaxation response?

For more than 30 years, Herbert Benson and colleagues, building on the work of Swiss Nobel laureate Dr Walter R. Hess, have described a physiological response, termed the 'relaxation response,' that is the opposite of the stress response [10]. It results in decreased metabolism, heart rate, blood pressure, and rate of breathing, as well as a decrease in brain activity [154].

The relaxation response appears to differ from the classical stress response in that the latter occurs automatically when one experiences stress, without requiring the use of a technique. In contrast, two steps are required to elicit the relaxation response: (1) the repetition of a word, sound, prayer, phrase or muscular activity and (2) when other, everyday thoughts intrude, there is a passive return to the repetition [6,52]. Many different methods can be used to elicit this learned relaxation response, including progressive muscle relaxation, meditation, autogenic training, yoga, and repetitive physical exercise. In addition, many forms of prayer can be used to elicit the relaxation response. The specific method used usually reflects the beliefs of the person eliciting the response [7]. The method may be secular or religious, performed at rest or during exercise.

Recent research has documented that regular elicitation of the relaxation response results in alleviation of many stress-related medical disorders. It is essential to understand that regular elicitation of the relaxation response also results in long-term physiologic changes that counteract the harmful effects of long-term stress throughout the day, not only when the relaxation response is being brought forth [52]. Relaxation-response based approaches, generally used in combination with nutritional, exercise, and stress management interventions, have been demonstrated to be effective in the treatment of hypertension [139], cardiac arrhythmias [9], chronic pain [22], insomnia [61,62], anxiety and mild and moderate depression [11], premenstrual syndrome [46], and infertility [33]. Because of this scientifically documented efficacy in a wide array of disorders, a possible physiological basis for many millennia-old mind/body Western and Eastern approaches has become more accepted.

Indeed, as a result of the evidence-based data, the relaxation response has become a part of mainstream medicine. Approximately 60% of US medical schools now teach the therapeutic use of relaxation-response techniques [43]; they are frequently recommended as therapy in standard medical textbooks and many family practitioners now use them in their practices. In this regard, we also surmise that the ability of a practitioner to elicit the relaxation response is strengthened by the trust or belief in the expected outcomes. In fact, the strength of the fiduciary relationship between physician and patient appears to play a direct role in the effectiveness of medical treatment [107]. Thus, it would appear that there is a physiological process at work, allowing for this response to occur. However, the exact mechanism or combination of cascading mechanisms involved until now has escaped detection.

4. What is the mind?

Before going further we must define the term 'mind.' For us, it was enticing to think that the chance alteration of genetic or neural pathways leading to cognitive processes also provided such endowed animals with an additional survival coping strategy [117] and that these cognitive coping abilities provided such organisms with a competitive edge for survival. The burgeoning of cognitive theory and therapy in the recent past is testimony to the insight that altering and improving cognitive coping mechanisms can help dissipate the emotional ravages attendant to the stress response.

In order for cognitive ability to develop and succeed, however, there must first be a unifying consciousness to control or regulate the many individual neural processes that potentially summate a decision-making process. That is, the brain represents only neural tissues organized into various neural patterns that can work together or separately. Without a unifying component being able to cope with a focus, the significance and uniqueness of this coping

strategy would be lost. These individual processes (storing sensory information and motor responses in many brain compartments, along with the multitude of simultaneous integration processes) are extremely complex and varied. Moreover, a unified entity, a 'mind', would only be involved with experience-related phenomena (both exteroceptive and interoceptive) since this is the realm in which coping strategies are designed.

We can also hypothesize that cognitive abilities arose or evolved because highly complex sensory-motor integration mechanisms were already in place (an organized brain) on which this 'highly developed' coping strategy could be based. In this regard, cognitive coping would be expected to use this foundation as its operating platform. That is, cognition must be able to activate normal non-cognitive stress phenomena as well as deactivate them at the appropriate time. Indeed, the subtleties of the integration would be hard to discern, as would the exact stimulus of a complex sensory experience. Yet, this would not take away from the existence of the connection/integration; it only further dramatizes its complexity. Thus, for example, we can predict that both cognitive and non-cognitive coping would be able to influence immune phenomena via neuro-immune, vascular-immune and neuro-vascular mechanisms. Based on the above, the mind depends on the underlying neural substrates to manifest itself. Thus, both consciously and unconsciously, it should be able to influence its underlying foundation.

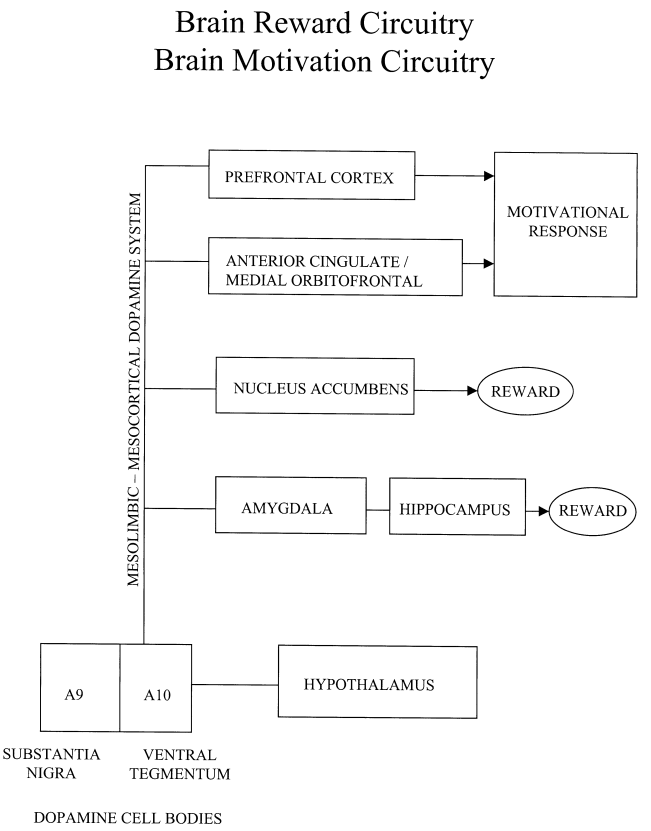

Fig. 2. Motivation and reward are mediated through the mesocortical–mesolimbic dopamine circuitry with cell bodies in the midbrain tegmentum, axons flowing in the medial forebrain bundle and terminal zones in the amygdala/hippocampus, nucleus accumbens, anterior cingulate/medial orbitofrontal cortex and prefrontal cortex. Memory of the pleasure of wellness and the pain of illness are accessible to this circuitry through hippocampal mechanisms. Belief affects mesocortical–mesolimbic appraisal of an experience leaving one stressed or relaxed. Belief in a placebo can elicit the relaxation response of remembered wellness with salutary effects on the stress response and immune response systems.

5. The mind–body link

5.1. Neurological

5.1.1. Emotion/belief and CNS–PNS wiring

Belief has an emotional component in that the brain motivation and reward circuitry will be reinforced with a positive emotional valence attached to the believed in person, idea or thing (see Fig. 2). This emotionalized memory replete with 'somatic markers', i.e., bodily sensations that accompany emotion and set the feeling tone, 'feels right' to the person [28]. Clearly, emotion can be viewed as a process reinforcing a belief so that rationality cannot 'weigh' the belief down into a lack of activity (see Refs. [116,117]). Indeed, belief in regard to a therapy and/or doctor and/or personal religion, may stimulate physiological processes, enhancing naturally occurring 'healthy' processes by augmenting their level of performance (Fig. 1). In this regard, emotional stresses (e.g., fear and anxiety) can induce cardiovascular alterations, such as cardiac arrhythmias [72,73,156]. These cardiovascular events can be initiated at the level of the cerebral cortex and may involve insular as well as cingulated, amygdalar and hypothalamic processes (Fig. 3). Clinically we may see this in elevated emotional situations that can induce sudden death in patients with significant coronary artery disease [104]. In addition, heart rate is often altered under stressful conditions. Neurons in the insular cortex, the central nucleus of the amygdala, and the lateral hypothalamus, owing to their role in the integration of emotional and ambient sensory input, may be involved in the emotional link to the cardiovascular phenomenon (Fig. 3). These include changes in cardiac autonomic tone with a shift from the cardioprotective effects of parasympathetic predominance to massive cardiac sympathetic activation [72]. This autonomic component, carried out with parasympathetic and sympathetic preganglionic cells via subcortical nuclei from which descending central autonomic pathways arise, may therefore be a major pathway in how belief may affect cardiovascular function. The importance of emotion (and therefore limbic activation) was further demonstrated in ischemic heart disease when patients with frequent and severe ventricular ectopic rhythms were subjected to psychological stress [72]. The frequency and severity of ventricular ectopic beats increased dramatically during emotional activation of sympa-

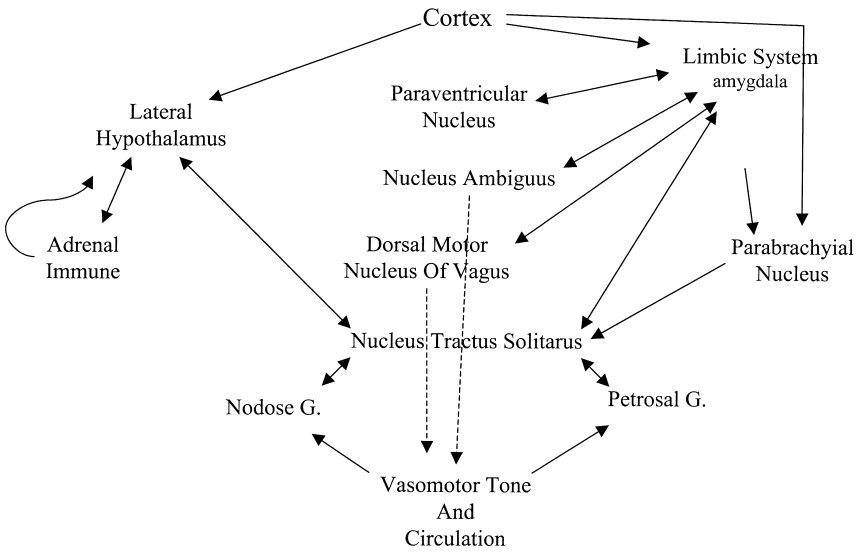

Fig. 3. Representative connections among the limbic–hypothalamic pituitary adrenal axis, demonstrating that these centers are linked to vascular tone regulation. This pathway suggests how mental and emotional phenomena may exert a level of top-down control on vasomotor activity and circulatory tone. The illustration is not meant to be all-inclusive.

thetic mechanisms but not during reflexively-induced increased sympathetic tone.

The hard-wiring of emotion/belief and cardiovascular neural systems involves many subcortical descending projections from the forebrain and hypothalamus [53–56,66,140]. (see Fig. 3) In 1875 cardiovascular changes were observed in experiments where the motor cortex surface was stimulated, eliciting tachycardia accompanied by and independent of changes in arterial blood pressure [103]. The 'sigmoid' cortex [23,103], frontal lobe [27,58,159], especially the medial agranular region [20], subcallosal gyrus [51], septal area [32,106], temporal lobe [74], and cingulate gyrus [74,108,149] appear to be involved. The insular cortex in cardiac regulation is important because of its high connectivity with the limbic system, suggesting that the insula is involved in cardiac rate and rhythm regulation under emotional stress [89,98,164].

Cardiovascular responses can be elicited by electrical stimulation of the hypothalamus [21]. In anesthetized cats, bradycardia and pressor responses are elicited on stimulation of the anterior hypothalamus and tachycardia and pressor responses were produced primarily by stimulation of the lateral and posterior hypothalamus [81]. Electrical stimulation of the anterior and dorsal hypothalamus may be dependent in part on the degree of basal vagal or sympathetic tone [21].

The amygdala, with respect to autonomic–emotional integration [29,63], is composed of numerous subnuclei, of which the central nucleus appears to play a major role in the elaboration of autonomic responses [78]. There are profuse inputs to this region from the insular and orbitofrontal cortices, the parabrachial nucleus, and the nucleus tractus solitarius [161,162]. Bradycardia and a decrease in respiratory drive are elicited when the central and lateral nuclei, the parvocellular part of the basal nucleus, the periamygdaloid complex, and the putamen are stimulated [16]. Amygdalo-tegmental projections are viewed as a critical link in cerebral cortical control of autonomic function [56]. The amygdala receives afferent connections from several areas of the cerebral cortex [1,64,98,161], including areas noted above through which cardiac rhythm can be affected.

The medial hypothalamus is also implicated in cardiac arrhythmogenesis [4]. Beattie and colleagues [4] suggested that hypothalamic projections that descended into the midbrain periaqueductal gray matter, reticular formation, and intermediolateral nucleus of the spinal cord mediate the response. Magoun and colleagues [77] demonstrated that the lateral hypothalamus and wide areas of the lateral tegmentum are also important for autonomic function. The lateral hypothalamus has long been recognized for its role in the regulation of motivation and emotion and the autonomic concomitants of related behaviors [102]. The densest cortical projection to the lateral hypothalamus arises from the infralimbic cortex [60]. Pressor sites within the insular cortex project more heavily to the lateral hypothalamus than do depressor sites and are represented at caudal levels [1,161]. Anatomical studies of the lateral hypothalamus demonstrate projections to the periaqueductal gray matter, the parabrachial region, parvicellular formation, dorsal vagal complex, and spinal cord [57,66,102,144]. Furthermore, descending projections of the lateral hypothalamus terminate as a capsule around the dorsal motor nucleus of the vagus nerve, which provides secretomotor fibers to the stomach wall, pancreas, and small intestine. These neural patterns might account for the close association of cardiac and gastric responses.

In the modulation of autonomic responses, the periaqueductal central gray has received greater recognition and its pathways involved in cardiovascular regulation and other functions have been identified [3,152]. The periaqueductal gray receives major projections from each of the cortical and subcortical areas discussed above and thus represents an important link for descending modulation of emotion, stress-related arrhythmias. The periaqueductal gray matter has also long been known to be involved in affective defense [26,59] and analgesia as a result of opioid processes [88]. The efferent projections of the periaqueductal gray are strongest to the rostral ventrolateral medulla, a region also show to contain endogenous morphine [119], including sympathoexcitatory fibers that project to cardiac sympathetic preganglionic neurons [150,151]. Thus, the heart rate can be influenced by lateral hypothalamic neurons that are sensitive to opioid peptides and their neural processes.

Given this, can a person's belief in an expected outcome actually effect the expected outcome? We believe the answer is yes, barring organic disease in the sensory, integrative or motor component of the processing pathway.

5.2. Psychological

Various laboratories throughout the world have sought to define the link between mind and body that they assumed was taking place. Recently, our group has also started to evaluate this relationship. In this regard, the relatively new field of neuroimmunology or psychoneuroimmunology brings together this scientific inquiry. Cognitive and non-cognitive neural processes originating in the brain/mind may communicate through simple signaling pathways to intimately link the central nervous system and the diffuse immune system, including vascular components (Figs. 1 and 3). While many of the body's processes occur without cognitive input, the use of cognitive intervention or awareness as a coping strategy allows us to manipulate non-cognitive processes, implying that the 'mind' can intervene and impose change in physiological systems. Indeed, this change can be both beneficial and pathological. Here we are concerned with the beneficial type of mind intervention since its role is to promote health and human longevity. Non-cognitive processes, therefore, may exist to promote health and cognition itself may stimulate these health-promoting mechanisms when properly activated or called upon.

Clearly this type of 'hard' non-cognitive and 'soft' cognitive linkage is instrumental in the concept of the mind–body phenomenon. We believe it is also quite important in understanding the placebo effect, since it may represent the physical manifestation of this phenomenon. That is, when a person who unknowingly receives a placebo thinks that the pill is going to work, the individual may invoke existing cognitive and non-cognitive processes (remembered wellness) based on this belief, that will then exert a beneficial effect on the outcome of a particular disease or disorder. Admittedly, these processes are called on in a very unconscious way, indicating that they may be of primal origin, in other words antibiosenescent. Learning to harness this process has been the goal of the Mind/Body Medical Institute.

In this regard, one of us (Herbert Benson), more than 30 years ago, realized that there are mechanisms/processes that may be called upon to initiate positive mind–body activation, whereby an individual can mentally focus and promote healing, depending on the viability of the existing physical mechanism. He referred to this process as the 'relaxation response'. We are now re-examining the mechanism of the relaxation response, in light of new knowledge that has been acquired by our laboratories and others. This speculative review constitutes an attempt not only to impose molecular and physiological parameters on the placebo effect, but also to understand one of the first attempts to control a phenomenon, the relaxation response, which is linked to it.

5.2.1. Stress

The term 'stress' must be defined because it is thoroughly interwoven in this proactive protective response of the relaxation response and the placebo effect. Stress, simply defined, represents an event or stimulus that alters the existing immediate organismic homeostasis or 'allostasis' [80]. Some theorists now refer to the 'healthy state' as one of allostasis or stability in the face of change. Multiple causes of stress add to what is called 'allostatic loading' which can be pathologic if not relieved. The state may be cognitively appraised or non-cognitively perceived. The 'disturbed' organism may either acutely or chronically experience this stimulus. Indeed, the stressor (the stimulus) may even emerge from within the organism itself, such as in interoceptive psychiatric stress. Stress is difficult to define because there are many types of stressors, or stimuli, that can bring on this homeostatic perturbation. Through an extremely complicated allostatic process, all living organisms maintain their survival in the face of both externally and internally generated 'stressors'. This apparent harmonization is constantly challenged often to the point of threat [24,42].

Following a stressor, survival is maintained within a steady state range by adaptational responses in a series of balancing and feedback activities reflecting an astounding array of biological, psychological and sociological behaviors. These behaviors are employed to cope with the effects of stress with the goal of reestablishing and/or maintaining allostasis. In this regard, counter-intuitively, stress responses can be viewed as being highly protective. The broad spectrum of stimuli capable of engaging the stress-response is remarkable and reflects how well integrated our perceptions of the physical, psychological and social worlds are [155]. In this context, 'stress' can be defined as a state of disharmony or allostatic loading that

threatens to overwhelm the organism's resources and strengths [24,80]. Biochemical (neurotransmitter, peptides, steroids), physiological (heart rate, blood pressure) and behavioral (anxiety, depression, tension) concomitants of stress may co-mediate a disease response [153].

As discussed above, stress responses can be seen as having a protective role. In order to be protective the response must rely on a broad array of reactions and cascades that are constitutively expressed. Indeed, if these critical stress-associated processes had to be induced through a slow response, for example, through transcriptional activation, the organism would cease to exist since the stress response must be a rapid protective response system. This concept is mirrored by our need for non-specific immunity and the rapid acute phase immune response to physical trauma, infection, etc.

Another important element of stressful stimulation may be the duration or time component of the noxious stimulus [42,110]. A brief physical or mental 'assault' may allow an organism, through various detailed allostatic compensatory mechanisms, to 'deal' with both an appraised or perceived stress. If the situation were to continue chronically, the organism might become vulnerable, susceptible to negative aspects of the stress response, such as in the case of prolonged immune down regulation [118–120,133–135]. Moreover, our physiological and psychological stress response 'systems' plainly function or were designed to function over the short term, not for prolonged periods of time.

The concept of stress that we share, physical disturbance, with all animals has been examined in a non-cognitive model system, the marine mussel, using electrical shocks as the stressor. In this model the organism is allowed under certain situations to respond by closing its valves to escape a noxious stimulus. If the animal is not allowed to escape the shocks, and they are repeated over time, only shocks delivered to the nervous elements cause the release of opioid-like substances which then activate immunocytes [115]. Thus, the importance of stress duration and resultant coping strategies appear to have evolved early during the course of evolution.

Is there evidence for evolutionary conservation of these strategies? We believe the answer is yes, especially, in light of signal molecule commonalities and similarities during the course of evolution, not to mention the common design of animal nervous systems regardless of phyla. The scientific literature contains many examples of this, strongly suggesting that the structure of signaling molecules, such as the neuropeptides and catecholamines, has been conserved throughout evolution. Recently, we have found proenkephalin-, prodynorphin- and proopiomelanocortin-like precursors in leeches and mollusks [99–101,126,127]. Given the fact that invertebrates (as described in these reports) contain adrenocorticotropin (ACTH), a neuropeptide of major significance in the mammalian stress response that functions to down regulate immune/defensive actions, strongly indicates this system evolved earlier than previously thought. The findings discussed above imply that intraorganismic adaptational processes to stressful stimuli are evolutionarily old. Furthermore, in animals 500 million years divergent from man, these stress-related responses are also rapid and protective.

The pathological effects of stress are those induced by long-term stress. This is what Hans Selye referred to as the 'general adaptation syndrome', when a particular stressor (highly emotional situation, physical abuse, etc.) remains for a long period of time [105]. Here the persistent elevated stress-response causes the system to function at its full capacity. This cannot continue for extended periods of time without metabolic detriment to the organism. Thus, short-term stress processes are beneficial because we can overcome a particular obstacle. Long-term expression of these processes can be viewed as detrimental. Our physiologic systems are not designed for long-term stress, such as prolonged immune compromise. We may also bring upon ourselves a long-term stress resulting from our perception of the stressor itself. Perhaps, with the appearance of cognitive appraisal capabilities human beings were, as a side effect, able to translate the short-term stress process into a longer-term stress process simply by thinking about it and moreover dwelling on it (such as contemplating a boss firing you for several months). Cognitive abilities have allowed us to appreciate stressors that may not be immediately apparent to anyone else, but may be internally apprised as such.

With chronic stress and the possible debilitation occurring in various physiologic systems, one can imagine that the mind is clearly inducing and may be propagating the effect. In this scenario, the mind becomes central to the process, as it does in the placebo and relaxation response. Interestingly, the ability to invoke pathways associated with long-term stress may depend on over burdening mind processes upstream and their neurological and signaling molecule links downstream. At the molecular level, signaling systems, including neuronal types, may become dysfunctional or desensitized to the constant elevated levels of particular signaling molecules. Counter-intuitively, the placebo effect may really represent a short-term stress, one that's designed to give a boost to the system over a short period of time once invoked. In this regard, both the placebo effect and relaxation response may also counter-intuitively share commonalities with stress processes (Fig. 1).

6. Constitutive proactive processes represent the placebo effect

6.1. Opioid peptides

As noted earlier regarding the placebo effect, we are looking for a naturally occurring proactive protective

mechanism that once stimulated, provides a beneficial outcome for the individual invoking the process. These processes must be constitutively expressed so that they can continually be 'felt' as well as be stimulated rapidly to increase their desired beneficial effect. One recent example of such a process comes from work done in our laboratory involving enkelytin [128] (Fig. 4). Enkelytin is a naturally occurring antibacterial peptide that is a proteolytic product of proenkephalin, a major opioid peptide precursor [47,48,82,138]. This precursor can also be processed into neuropeptides such as met-enkephalin that can alter brain function, in reward and analgesia pathways (Fig. 4). Enkephalins have also been associated with a sense of well-being (see Ref. [128]). Furthermore, enkephalins can stimulate immune cells, enhancing their functions [135]. Interestingly, upon further processing, enkelytin liberates met-enkephalin-arg-phe that can also further stimulate immune cells(Fig. 4). In addition, in the mammalian central nervous system, when enkephalinergic neurons release this opioid peptide, they may be liberating the antibacterial peptide as well [128]. Therefore, the molecules that can induce a sense of well-being can also enhance immune function, while simultaneously producing an antibacterial peptide. This represents, therefore, an enhanced innate protective mechanism that is constitutively expressed (see Refs. [128,130]). Furthermore, this process can be found in both invertebrates and in humans undergoing major surgery [142,143].

The association of a mood-enhancing and a reward-type molecule with both antibacterial and immune-cell-stimulating functions represents an important link within the mind–body phenomenon. Again, these compounds are present simultaneously, so when we are feeling good we have created a naturally occurring antibacterial state. Furthermore, opioid peptides have been implicated in the stress response, including the induction of proinflammatory signals [135,166]. How widespread is this association between a sense of well-being and antibacterial actions? The enkelytin molecule found in invertebrate organisms that evolved 500 million years before man exhibits 98% sequence identity to the protein expressed in mammals [100]. Thus, we propose that this is part of the underlying proactive protective mechanism that has evolved and that can be associated with immune and nervous system allostasis.

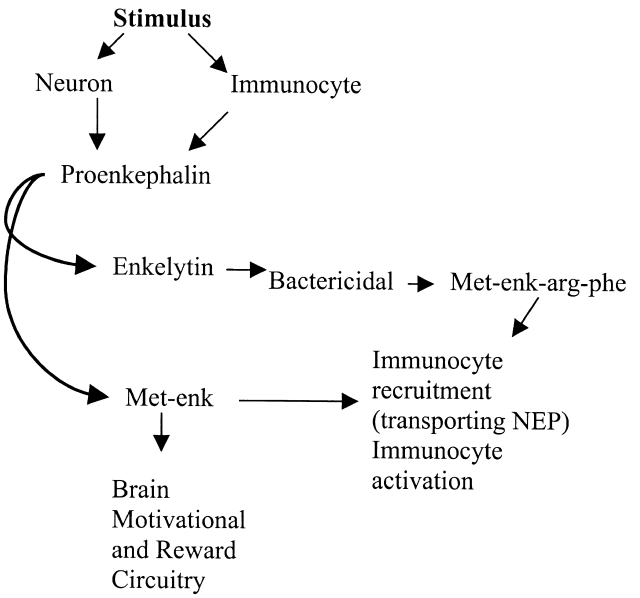

Fig. 4. A disturbance, trauma event, or antigenic challenge may stimulate the processing of proenkephalin A in either a neuron, immunocyte or both liberating enkelytin, an antibacterial peptide and methionine enkephalin. The latter can stimulate immune cells and/or, if in a specific area of the central nervous system motivational and reward circuitry. Enkelytin can be further processed into methionine enkephalin-Arg-Phe, which also has immune stimulating actions see Ref. [128].

Interestingly, there are other naturally occurring antibacterial peptides that are expressed in mammals, such as chromogranin-A, -B, and secretogranin [47,48,82,138]. What is the purpose of this proactive antibacterial peptide response? We need these peptides because they are naturally invoked to protect us on a moment-by-moment basis from our greatest threat, microbes. Can we, however, cognitively invoke these mechanisms to enhance our health?

The antibacterial peptides discussed above have been found in adrenal chromaffin cells as well as in the hypothalamic–hypophyseal system [47,48,82,138]. Thus, they are strategically located in anatomical areas classically associated with the stress response. This common localization causes us to speculate that short-term stress may really elicit the proactive protective unified response associated with the placebo effect. Short-term stress is likely to be associated with the release of enkephalins and the antibacterial peptides as noted above. We hypothesize that short-term stress would also release antibacterial peptides that would innately protect the organism over the short term. Furthermore, belief in a doctor or pill may actually produce this innate protective response [107].

In a recent study, we examined expression of antibacterial peptides in both invertebrate tissues and humans undergoing coronary artery bypass surgery [142,143]. In invertebrates, 15 min after an insult to the tissue, such as cutting of the surface of the animal's skin, we began to see the simultaneous appearance of enkephalin and enkelytin. In human surgical patients undergoing major surgery, 15 min after the initial incision the same two molecules appear. Here, we have observed a very interesting and conserved mechanism demonstrating that these molecules appear without conscious thought, and suggesting that they represent an underpinning of processes that work to protect us from microbial invasion. Can we, however, invoke these processes when they are needed?

With regard to CNS-mediation of the appearance of antibacterial peptides following coronary artery bypass surgery, we propose in an analogy with invertebrate tissue,

that the surgical incision represents the stress stimulus. Chromaffin cells are innervated by preganglionic fibers conveyed by the splanchnic nerves which pass through coeliac and renal sympathetic nerve plexuses and are under the control of stress-sensitive supraspinal centers in the brain (see Ref. [2]). Furthermore, the peptides we identified are contained within chromaffin granules in cells of the adrenal medulla [82]. Thus, the increases in peptide levels we observe may be due to release of adrenal chromaffin granules caused by a systemic stress response [84]. Additionally, PACAP-containing neuronal cell bodies located in sensory neurons of dorsal root ganglia can be activated by sensory receptors located in the skin [84], suggesting that incision may cause the release of these peptides from the adrenal medulla. In either event, both opioid peptides and antibacterial peptides can be released simultaneously.

6.2. Nitric oxide

In this regard, we have also examined nitric oxide (NO) signaling, since it has a constitutive component (constitutive NO synthase (cNOS) endothelial (e) and neuronal (n) isoforms; see Ref. [118]). Constitutive NOS (cNOS), as the name implies, is always expressed. When cNOS is stimulated, NO release occurs for a short period of time, but this level of NO can exert profound physiological actions for a longer period of time [30,118]. NO is not only an immune, vascular and neural signaling molecule, it is also antibacterial, antiviral and it down-regulates endothelial and immunocyte activation and adherence, thus performing vital physiological activities, including vasodilation [112,118]. Thus NO, within the concept of the placebo effect, has the potential to protect an organism from microbes and physiologic disorders such as hypertension, and also diminishes excessive immune and endothelial activation [112,118]. Indeed, its continued presence may set the tone for the activation of these cells and its absence or presence at lower levels may set the stage for progressive deterioration, i.e., Alzheimer's Disease (see Refs. [30,118]).

The endocannabinoids, anandamide and 2-arachidonyl glycerol, are naturally occurring cNOS-derived NO-stimulating signaling molecules that are also constitutively expressed [31,35,36,79,93,111,113,118,121,125,129,131,132]. Anandamide, an endogenous endocannabinoid, can also cause NO release from human immune cells, neural tissues and human vascular endothelial cells [31,123,124]. Anandamide can also initiate invertebrate immune cell cNOS-derived NO [121]. Estrogen can also stimulate cNOS-derived NO in human immune and vascular cells [114,123,124]. Why are there so many pathways that lead to cNOS-derived NO release? We believe that each signaling system performs this common function under different circumstances. Morphine, another naturally occurring animal signal molecule [119],given its long latency before increases in its levels are detected, arises after trauma/inflammation and, through a NO mechanism, down regulates these processes in neural and immune tissues [111,133,148]. Anandamide, as part of the ubiquitous arachidonate and eicosanoid signaling cascade, serves to maintain and augment tonal NO in vascular tissues [35,118]. Estrogen, through NO release, provides an additional pathway by which the system can down-regulate immunocyte and vascular function in women [123,124]. This may be due to both the immune and vascular trauma associated with cyclic reproductive activities, such as endometrial buildup, when a high degree of vascular and immune activities are occurring. Given the extent of proliferative growth capacity during peak estrogen levels in this cycle, NO may function to enhance down-regulation of the immune system to allow for these changes. Clearly, therefore, enhanced cNOS activity would be a beneficial effect within the concept and time framework of the placebo effect and the relaxation response.

6.3. Signaling molecules in the relaxation response

In this phenomenon (as noted above), once individuals undergo a very mild form of work such as meditation, e.g., relaxation response, they experience peripheral vasodilation, warming of the skin, a decrease in heart rate and an overwhelming sense of well-being. Indeed, as the name implies, the relaxation response appears to be part of our ability to relax and or recover. In this regard, at first glance, we may be countering the effects of prolonged stress. The relaxation response is also mediated by signaling molecules as suggested by the fact that norepinephrine is modified [52]. Regarding the vasodilator peripheral heat-warming processes, we speculate that this involves NO. In regard to the sense of well-being we can assume that this process may also involve opioid signaling molecules.

In examining a potential mechanism for the relaxation response, besides the over-riding central nervous system output via the autonomic nervous system, peripheral neuro-vascular processes would appear to be important (Figs. 1 and 3). We surmise NO to be of fundamental importance in this response because of the increase in peripheral temperature, i.e., vasodilation [139]. However, the vascular peripheral control mechanisms involved with the regulation of NO have yet to be deciphered. Nevertheless, in examining the peripheral vasculature we find nerve terminals in the vessels that when stimulated by nicotine, result in vasoconstriction followed by vasodilation [86], indicating a cholinergic mechanism. Clearly, this phenomenon is in line with the past description of the relaxation response [52,61]. The vasoconstriction component of the biphasic nicotine effect is mediated by α_1 adrenoceptors stimulated by norepinephrine (NE) liberated from peripheral sympathetic adrenergic nerves (Fig. 5). Studies suggest that because of insensitivity to atropine, acetyl-

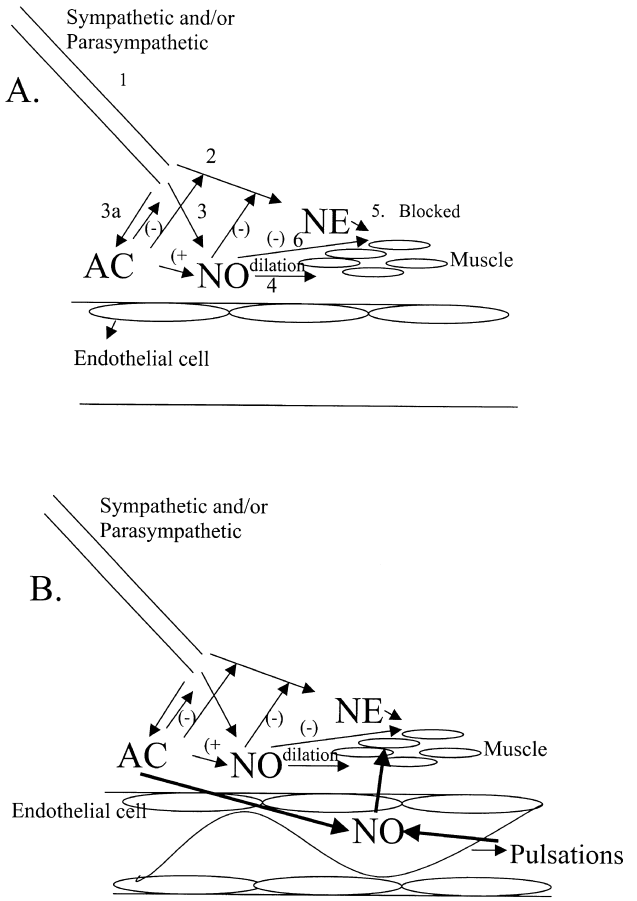

Fig. 5. Diagrammatic illustration of the hypothetical manifestation of the peripheral relaxation process. (A) Neural contribution to the peripheral relaxation response mechanism. Both sympathetic and parasympathetic nerves (combined as 1) innervate an arterial blood vessel using norepinephrine (NE; 2) and acetylcholine (AC; 3a), respectively. Additionally, a nitric oxide (NO; 3) transmitting nerve also is present in close association with the sympathetic component of the vessel innervation. In this scenario, ACh via M2 receptors can inhibit NE and NO release. NO can also inhibit NE release and synthesis. No release initiates smooth muscle vasodilation via cGMP. Interestingly, reports noted in the text demonstrate that this innervation can first produce vasoconstriction followed by vasodilation, exactly the same scenario that takes place in the relaxation response performance. We surmise the simultaneous release of NE and NO accounts for this phenomenon, since in the presence of NO, NE cannot sustain vasoconstriction [122]. Thus, there is a delicate balance occurring between ACh, NE and NO. This balance is probably mediated via CNS interaction (see earlier figures) the results converging on these peripheral vascular elements to achieve an initial constriction followed by a more pronounced dilation. Clearly, this balance encompasses the process whereby vasoconstriction is not followed by vasodilation, i.e., NE effect occurs unhindered. (B) Given the delicate nature of this balance between central and peripheral expression of the relaxation response, we also surmise that peripherally produced NO may be involved in modulating this mechanism. Here we note that normal pulsations, via heart induced pressure fluctuations, also enhances the basal level of eNOS derived NO as does ACh on the vascular endothelium [13,83,94,118]. In this circumstance the additional or complementary NO may further contribute to the ability of NO to interfere with smooth muscle contraction as well as NO to inhibit the release and synthesis of NE [31]. Thus, at the present time, we can demonstrate that enough is known about the relaxation response in that a peripheral regulatory mechanism can be proposed. Furthermore, as in many physiological regulatory processes these events may differ in different tissues.

choline (ACh) does not mediate the nicotine-induced vasodilation [86]. Instead it is mediated from nerve endings in which a NO generating system and ACh may coexist [163]. Thus, nicotine stimulates the adrenergic and nitroxidergic nerves innervating, for example, the temporal arterial wall of denuded endothelium in superficial dog tissue, resulting in contraction and a rapidly developing relaxation, the latter being mediated by cGMP [145]. Slow relaxation caused by nicotine is associated with the elevation of cGMP production via activation of guanylate cyclase, which appears to be mediated by prostaglandin I_2 [145].

Taken together, we surmise that NE initially promotes a slight vasoconstriction of the artery, indicating a slight enhancement of sympathetic activity upon stimulation. This is immediately followed by the release of NO from the nitroxidergic nerve, which mediates a concentration dependent vasodilation. In monkeys, the cerebral arterial diameter under resting conditions is maintained by tonic release of NO from the nerve (10–20%) or from the nerve and endothelium (30%) [146]. This observation is supported by other data from our laboratory since basal NO is cNOS-derived and keeps particular types of cells in a state of inhibition [118]. Endogenous superoxide dismutase (SOD) in the cerebral artery appears to protect the relaxation induced by NO from perivascular nerves from the NO scavenger action of superoxide anion [141]. This NO then produces the longer-lived phenomenon of smooth muscle relaxation. In another report, it was found that NE vascular hyper-responsiveness in hypertension is dependent on an impairment of NO activity that is realized through norepinephrine-induced oxygen free radical production [69], demonstrating an important contribution to the understanding of this regulatory process.

The location of the NO releasing nerve, nitroxidergic nerve, has continued to be a subject of debate. Various findings lead us to believe that the nitroxidergic nerve is located in the proximity of the adrenergic sympathetic nerve bundle. Furthermore, the NE-stimulated vasoconstriction is followed by relaxation that is suppressed by L-NA, a NOS inhibitor [165], supporting its interaction via a NE mechanism [68]. Secondly, nicotine-induced relaxation is abolished by guanethidine, tetrodotoxin, and pretreatment with 6-hydroxydopamine, all causing destruction of the sympathetic nerve, and demonstrating again the NE component [147]. Furthermore, we have demonstrated that eNOS derived NO can inhibit NE neural vascular release [31]. Recently, we have also demonstrated that once NO is present, smooth muscle cells from rat and human arteries fail to contract in response to NE [122], demonstrating that once the balance shifts to NO, NE cannot initiate vasoconstriction. Thus, the NE reported in the relaxation response [52] may, in a sense, represent the left over *flow* of the earlier mild sympathetic stimulation, i.e., also representing *work initiation*.

Complicating this matter is the data indicating that ACh

inhibits, acting via prejunctional muscarinic receptors, the synthesis and release of NO while concurrently antagonizing the release of NE [87,146]. However, the response to exogenous NO is not influenced by ACh [141]. In addition, the secondary vasodilation to electrical nerve stimulation appears to be attenuated by treatment with ACh in a concentration dependent manner [141]. These findings suggest that ACh plays an essential role in vascular NO regulation. In this regard, it may be the factor adjusting the interaction of NO and NE, simultaneously exerting its own vascular action, i.e., stimulating endothelial NO release [44,45]. Others found that β_2-adrenoceptor antagonists blocked the relaxation induced by nicotine. Furthermore, they demonstrated that β_2-adrenoceptor immunoreactivities and NADPH diaphorase reactivities were colocalized in the same nerve fibers in basilar and middle cerebral arteries [68]. The authors speculate that NE acts on presynaptic β_2-adrenoceptors located on the NOergic nerve terminals to release NO resulting in vasodilation.

We also surmise, based on current studies, that endothelial derived NO, released through normal pulsations, due to vascular dynamics responding to the heart beat [13,94,118] as well as ACh stimulated endothelial NO release, may contribute to the effect of NO in inhibiting NE processes as well as inducing smooth muscle relaxation (Fig. 5). Furthermore, vascular pulsations may be of sufficient strength to also stimulate nNOS derived NO release, limiting any basal NE actions. Interestingly, nitrosative stress, mediated by involvement of the reactive nitrogen oxide species, N_2O_3 does inhibit dopamine-β-hydroxylase, inhibiting NE synthesis and contributing to the regulation of neurotransmission and vasodilation [34].This system may provide an autoregulatory mechanism involved in the neuronal control of peripheral vasomotor responses.

In conclusion, the relaxation response peripherally appears to be mediated by a system of regulation involving NO, NE and ACh as neurotransmitters and local hormones. Contingent on the preliminary vasoconstriction and depolarization of the membrane initiated by the release of NE, vasodilation is mediated by NO liberated from vasodilator nerves that activate guanylate cyclase in smooth muscle and produce cGMP. During this stage, NO and NE exist simultaneously. Due to the characteristics of NO, NE no longer mediates vasoconstriction; instead NO activates guanylate cyclase, which produces vasodilation and the relaxation response under a depolarized membrane state.

The central nervous system regulating pathways and processes integral to mediating this process stimulates NE release either alone or in combination with NO. If NE is released alone, vasoconstriction occurs. If NE is released with NO, we surmise an initial short-lived vasoconstriction occurs followed by a prolonged vasodilation mediated directly by NO. In this regard, if basal/tonal NE is present NO overrides this effect. If ACh is present, we surmise that its inhibition of NE, causing loss of sympathetic tone, and its stimulation of endothelial derived NO, may also result in vasodilation. The important point at this stage of explaining the relaxation response is that, at last, we are beginning to see a mechanism that can explain its characteristics and simultaneously provide an explanation for its health benefits, i.e., via cNOS-derived NO (see Refs. [30,118]).

7. The hypothesis

Given the antibacterial peptide, enkephalin and NO signaling pathways mentioned above, as well as the large number of other constitutive processes that have the potential to impact on the placebo effect, we must ask: is there a link between these processes? We know that these molecules and their signaling systems are important because they have been conserved, and in many cases their functions have also been conserved, from simple animals to man. We believe that these signaling molecules are involved in the short-term stress/relaxation response and also in the placebo effect.

If we look at the molecular mechanism of action of molecules like the enkephalins, we find that they have the ability to control the release of other signaling molecules, such as NE. This regulation of synaptic release occurs in brain and vascular tissues. Additionally, we also know that NO, which is liberated from many tissues such as nervous, endothelial, and immune cells, also has the ability to regulate neurotransmitter release and serves as a signaling molecule itself. This link is further evidenced by the fact that opioid and endogenous opiate signaling molecules may use NO as the mechanism to effect physiological systems. These signaling systems, therefore, may exemplify a conserved mechanism that has the ability to be stimulated over a short period of time and may become desensitized over a long period of time. The detrimental effects of prolonged stress may, therefore, be related to the sustained actions of this system, while the short-term actions of these signals may provide a quick ‘boosting’ effect that may be related to or actually involved in the placebo effect that we defined earlier.

Given this, we begin to associate cNOS-derived NO with the early or prime events that lead to the relaxation response because it is this quick and temporary increased release of NO beyond the basal level that will lead to vasodilation and peripheral sense of warmth. Its presence can also explain the paradox of the presence of norepinephrine in plasma while vasodilation is taking place [52].

Many immune processes perpetuate and become embellished with time by the recruitment of cells, and through beneficial yet sometimes harmful signaling molecules such as the pro-inflammatory cytokines. These molecules can all be down regulated by morphine, which is released following stress or trauma [119,135], specifically through cNOS-derived NO under certain circumstances. Thus, morphine

may help overcome over-stimulated immune and neural tissues. As such, it may be part of the immune system regulation that prevents the all too common ravages of what Bone called 'immunologic dissonance', including the systemic inflammatory response syndrome (SIRS) which sometimes culminates in the often lethal multiple organ dysfunction syndrome (MODS) [15]. It is possible that some individuals may be deficient in this regulatory process, leading, when challenged, to the unregulated, potentially damaging immune responses of SIRS and MODS. We have found, for example, that in the immune disorder histiocytic medullary reticulosis or malignant histiocytosis [41] a morphine selective receptor, μ3, was not expressed, and granulocytes and monocytes cultured from this patient could not be down regulated when exposed to morphine. The foundation of the concept that morphine is critical to a relaxation or a down-regulation of excitatory processes is supported by these preliminary clinical findings.

8. Molecular mechanisms

The physiological significance of cNOS-derived NO is that it can influence proinflammatory and stress situations, presumably bringing both the 'acute phase response' and the 'acute stress response' under control [30,111,118]. We propose that cNOS-derived NO initiates these events in part by its ability to modify the function of the transcription factors, i.e., NF-κB [157] (Fig. 6). NF-κB binding sites are present in the promoter regions of proinflammatory genes such as tumor necrosis factor (TNF), interleukin (IL)-1 and IL-6 (see Ref. [157]). Interestingly, stimulation of cells with proinflammatory cytokines leads to the degradation of the NF-κB inhibitor IκBα, liberating NF-κB. NF-κB is then free to activate the transcription of some of these very same proinflammatory cytokines. NO inhibits the expression of proinflammatory cytokines by stabilizing the NF-κB–IκBα complex [157] therefore preventing translocation of NF-κB into the nucleus. NO can also interfere with the binding of NF-κB to the promoter region of pro-inflammatory cytokines such as IL-6, which causes T-cell proliferation [157]. Perhaps this is why a correlative association has been found between higher levels of IL-6 and low church attendance, indicating a perturbation in a belief process [65]. Therefore, we speculate that specific stimuli such as belief and religious behavior may, given there worth to individuals, initiate cNOS NO release and may, as a result, prevent the expression of potentially deleterious cytokines. If a proinflammatory condition were uncalled for, its inhibition would tend to have great significance. Lack of such inhibition could also, for example, predispose one to autoimmune disease. In addition, our experiments suggest that cNOS-derived NO also exerts a tonal inhibitory action on NF-κB activation [30,118,157].

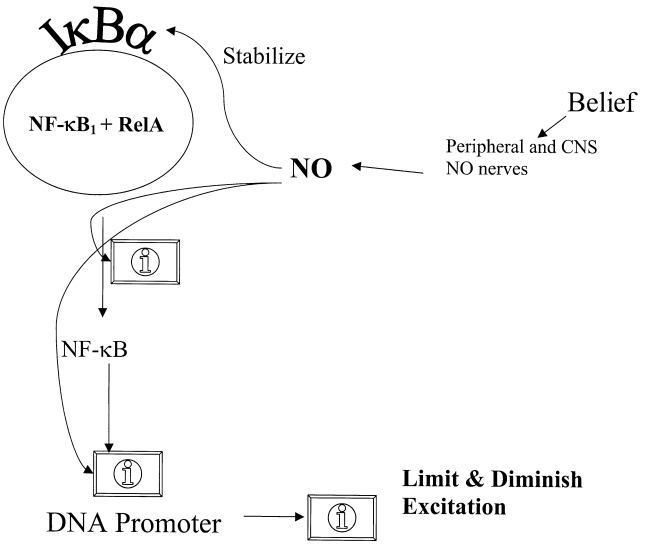

Fig. 6. Representation of NF-κB activation and its inhibition by nitric oxide via a 'belief' mediated pathway (see earlier figures). Thus, belief may alter stress perception or performance in such a may that the NO system predominates (Fig. 4). Briefly, excitatory cytokines, for example, can promote the disassociation of inhibitor IκBα liberating NF-κB that then translocates into the nucleus to bind to specific sites on DNA promoter regions. These DNA promoter regions are coding for proinflammatory cytokines, i.e., IL-6. This binding is then associated with the activation and expression of these proinflammatory associated molecules. Agents that stimulate constitutive nitric oxide synthase (eNOS) liberating nitric oxide (NO) may limit the degree of NF-κB activation, i.e., estrogen, since NO would inhibit (i: in box) the disassociation of the IκBα inhibitor complex, NF-κB binding to the respective DNA promoter region and the subsequent expression of the proinflammatory cytokines. In short, belief may actually diminish excitatory cell levels, i.e., vascular and immune [30,76,118,119,122].

Putting this in perspective, if we liberate molecules that have the ability to stimulate cNOS, producing a quick burst of NO, we can enhance the inhibition of NF-κB activation, limiting the extent and the severity of a pro-inflammatory situation. Does this occur during long-term stress or does this mechanism become desensitized and over stimulated by a relentless insult so that NO is formed through inducible NOS, which not only becomes detrimental to the cells, but to the entire organism? We believe the latter situation emerges with chronic stress and may give rise to certain degenerative diseases [40]. For example, if a proinflammatory challenge is overwhelming or persistent, iNOS production may reflect a last ditch attempt to overcome antigenic stress while also trying to dampen a potentially destructive pro-inflammatory blaze. The resulting overproduction of NO is unfortunately toxic.

Perhaps we see an end stage example with acquired immunodeficiency syndrome (AIDS). In this devastating disease infected macrophages set in motion an acute phase response which becomes chronic in the face of relentless HIV invasion [71,137]. This invasion is especially destructive in that CD4 T-cells are preferentially destroyed in the midst of a pro-inflammatory response, leaving the host

immunodeficient in a particular way and subject to overwhelming infection. The infected person winds up with the worst of situations, since in addition to immunodeficiency, there are the ravages of the immune response itself, in that there is an over production of NO by macrophages, microglia and astrocytes leading to neurotoxicity and the neurodegeneration of AIDS dementia [71].

Stress has been shown to play a role in the progression of human immunodeficiency virus (HIV) infection to AIDS. Most recently Leserman et al. [70] report that faster progression to AIDS is associated with higher cumulative average stressful life events, coping by means of denial, higher serum cortisol and lower cumulative average satisfaction with social support. It may be that the combination of a persistent inflammatory HIV vector and a persistent stress vector summate to produce an overabundance of iNOS with an outpouring of toxic NO. There is pathogenicity in the setting of a chronic phase response and a chronic stress response.

NO itself has the ability to up-regulate enzymes [130] such as neutral endopeptidase 24.11 (see Ref. [130]). Immunologists refer to this enzyme as the acute lymphoblastic leukemic antigen (CALLA) or CD10. Interestingly, neuroscientists refer to the same enzyme as enkephalinase. As the name implies, enkephalinase has the ability to process proenkephalin and, interestingly, it is expressed in humans as well as in invertebrates [130]. NO stimulates enkephalinase leading to the release of the proteolytic products, i.e., enkelytin as well as enkephalin [130]. Thus, NO can control and regulate enzymes that are responsible for liberating these crucial molecules that have a proactive protective function.

An important question remains: What causes the initial NO release? If you look at the relaxation response you quickly realize that this is a process that takes place over minutes (for example, you feel warm within minutes due to vasodilation after starting meditation). The stimulus that invokes this release must be of neural origin, implying that the neurovascular processes themselves are at the heart of this communication.

The significance of this new concept of neurovascular involvement was made evident recently by the demonstration that morphine, anandamide and estrogen can stimulate NO release in the vasculature of median eminence fragments affecting hypothalamic neurosecretion [91–93]. This is significant because median eminence NO causes the release of gonadotropin releasing hormone and ACTH via CRF, a molecule well established in stress responses. Naturally occurring molecules, therefore, have the ability to stimulate cNOS-derived NO release that can mediate vasodilation, antibacterial and anti-viral activity, signal molecule release and inhibition of immunocyte adherence to the endothelium.

Supporting this concept of cNOS-derived NO in the placebo effect are studies involving estrogen. Macrophages and granulocytes that can release NO have been found in atherosclerotic lesions, suggesting their involvement (see Ref. [38]). Estrogen-stimulated cNOS-derived NO down-regulates these cells, underscoring the significance of estrogen signaling as a hematological and vascular phenomenon [123,124]. In this regard, a positive correlation has been found between plasma 17β-estradiol and levels of stable metabolites of NO during follicular development in women [96]. Consistent with a role for NO, endothelium-dependent coronary artery vasodilation is increased after 17β-estradiol treatment in ovariectomized monkeys [158] and post-menopausal women [25,49,50]. NO may also function to protect blood vessels against atherosclerotic development by inhibiting immunocyte adhesion to the endothelium (for review, see Ref. [38]). These studies support our view that estrogen is part of the placebo mechanism; they imply that estrogen's presence augments/enhances the down regulating aspect of NO by increasing NO levels. Thus, neural processes that stimulate estrogen release, may provide the initial stimulus for immune and vascular beneficial effects by limiting the degree of the respective tissues to be excited (see Refs. [30,118]).

In 1996, we published a paper demonstrating that in patients who were just about to undergo surgery, their ACTH levels decreased [39]. This observation is clearly counter intuitive to the expected increase, for in the classic stress response one would expect that in these individuals who are going to undergoing major surgery that ACTH levels would rise. Examining this phenomenon more closely, we found that neutral endopeptidase levels were increased in these patients. This led us to suggest that signal systems were operating, i.e., NO, to first enhance neutral endopeptidase activity that would then process ACTH. Following this initial event the signal molecule level would rise via a positive end-product feedback loop that includes low ACTH levels [136]. Thus, the drop in ACTH levels may constitute the trigger to induce the further formation of its precursor, proopiomelanocortin. Therefore neutral endopeptidase, triggered by the stress release of cNOS-NO, can be seen to be involved in this stress regulation process. This links the stress phenomenon even closer to the NO model that we are examining and proposing.

In speculating on an overall theory as to what constitutes the relaxation response, we offer the following hypothesis: neural processes activate downstream signaling molecules that stimulate cNOS-derived NO release from immune, neural and vascular tissues. Prior to NO release this process also invokes the release of NE and opioid peptides. The presence of NO is deduced by its vasodilating actions and the lack of vascular sensitivity toward NE. This also helps explain the paradox of NE's increase and the lack of vasoconstriction during the relaxation response [52].

The placebo effect can also be explained within this context. Neural processes lead to the release of NO, stimulating neutral endopeptidase activity and causing the metabolism of proenkephalin and enkephalin, and the

liberation of antibacterial peptides. Simultaneously then, the released NO can exert its anti-viral, anti-bacterial and immune and vascular properties. In comparing the relaxation phenomenon with the placebo effect, we basically have the same mechanism in action, however relaxation techniques designed to elicit the relaxation response can be cognitively learned while the placebo effect route to the relaxation response is, at best, conditioned. Hence we may invoke the placebo effect via the relaxation response because the underlying mechanism may be within our cognitive control.

Recently Lazar and colleagues [67] reported that the relaxation response achieved by experienced meditators while undergoing functional magnetic resonance imaging (fMRI) was significantly correlated with activation in the hypothalamus, midbrain, hippocampus, amygdala, anterior cingulate and other areas in the brain motivation and reward circuitry. (see Figs. 2 and 3) These same areas are known to be under modulatory control of met-enkephalin. The sense of well-being that accompanies the relaxation response and the remembered wellness of the placebo effect may, therefore, involve the met-enkephalinergic modulation of these aminergic systems [14,67]. Again, this relationship between opioid and aminergic signaling has a long evolutionary history (see Ref. [109]).

An important question to ask is: why do we not find evidence for the placebo effect working all the time? Why is it just associated with a ‘sugar pill’? The answer, probably, is that it *is* working at a basal level all the time. Indeed, it is probably this process along with many others that provide for mammalian longevity, i.e., it is antibiosenescent. Its expression probably also differs among individuals, and the strength of one’s beliefs may also exert a profound impact on the heightened expression of these proactive innate protective responses that originate from the central nervous system. Therefore, the ‘sugar pill’ may

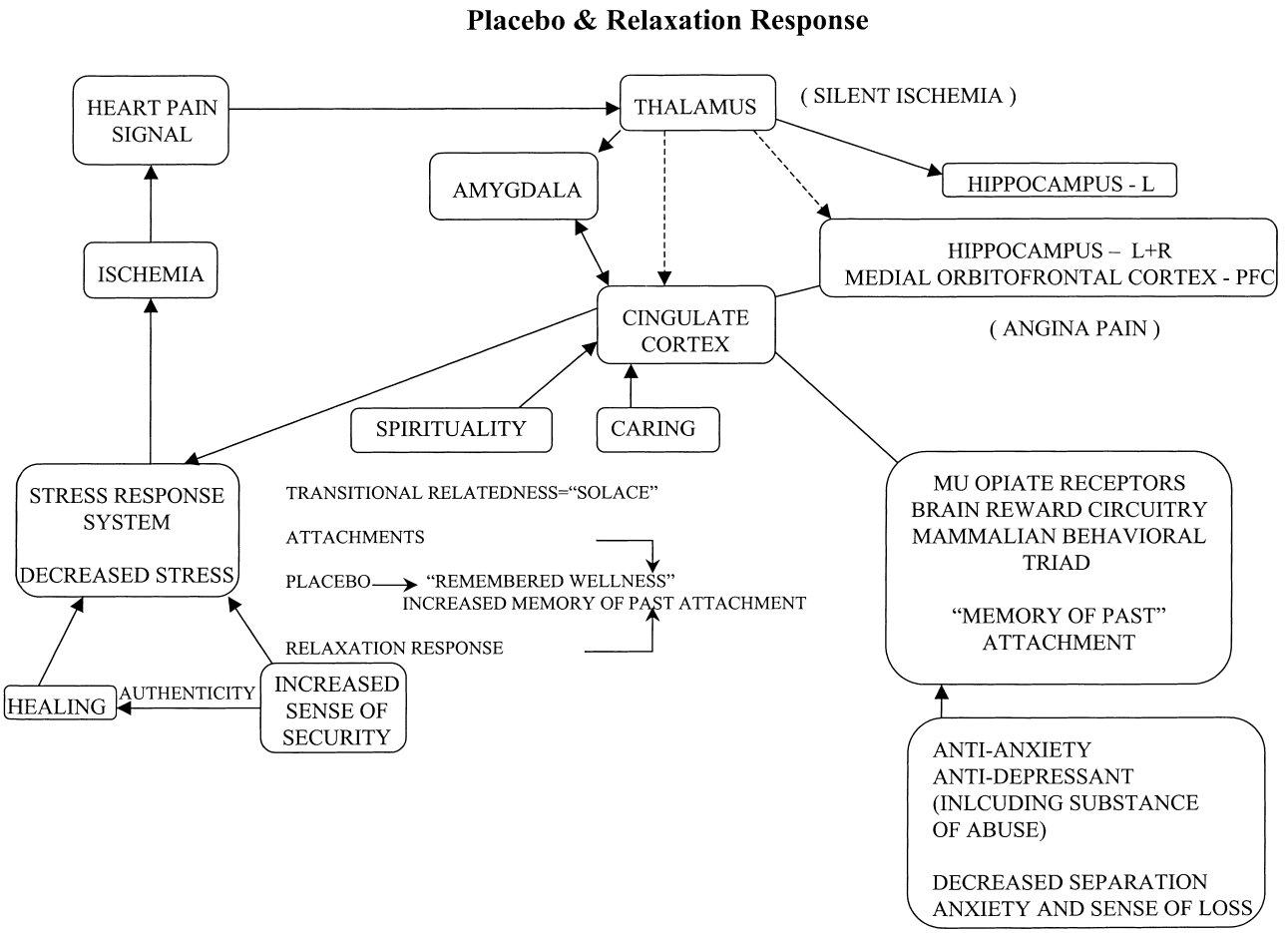

Fig. 7. It is now known that mental stress can elicit myocardial ischemia [97] leading to production of an anginal warning. In order for that anginal warning to be perceived and not become a case of silent ischemia, the signal must make it past the thalamus [95] and into the mesocortical–mesolimbic brain reward circuitry according to recent positron emission data [67]. This region also mediates our memory of past attachment — the ultimate in ‘remembered wellness’ [8] — signified in the mammalian behavioral triad of maternal nurturance [75], the separation cry to solicit attachment [18,160] and social play. Physician caring, certain medications, the placebo effect, the relaxation response and spirituality all can access the healing power of the solace [85] of attachment, decreasing stress response and immune toxicity and thereby potentially reducing ischemia. This may distally be related to a constitutive nitric oxide mechanism set in motion proximally by neural processes. LC-NE, Locus coeruleus–norepinephrine; PVN, paraventricular nucleus; CRH, corticotropin releasing hormone.

represent another cognitive link alongside the relaxation response to enhance the expression of this process as well as the strength of its expression, suggesting that cognitive links do exist in immune modulation. However, the placebo effect and relaxation response take place in that emotionalized transitional relationship between patient and doctor where the healing power of trust and hope are felt more than they are conceptualized. This attachment-based solace provided by a doctor's care may manage to have a salutary effects on the limbic neural processes in the brain motivation and reward circuitry responsible for regulating the stress response and immune response systems via constitutive NO pathways [37,85,118]. In Fig. 7, we present a theorized version of how these forces are at play in the human experience of cardiac disease.

Secondly, we must ask another question: why does this placebo effect not work under all pathological circumstances? One answer is, in all probability, that some pathological processes may overwhelm, diminish or halt the proactive protective pathways themselves, such as in the case of the individual with histiocytic medullary reticulosis where the μ3 opiate receptor was not present, so morphine could not inhibit the delinquent excitatory immunocyte behavior regardless of the individual's belief processes. This also demonstrates that placebo processes are organically based.

This speculative review has not been designed to answer all questions in regard to the relaxation response or the placebo effect. However, it does attempt to access these phenomena in light of current knowledge. In this regard, we conclude that there is a scientific basis for their presence. We further predict that an even greater number of physiological and biochemical processes will be found that will offer an even greater understanding of their operation. Finally, it is by way of this research, that we may be able to harness the body's own 'healthy' processes, allowing us to live longer and healthier lives.

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