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Noradrenergic Mechanisms in Stress and Anxiety: I. Preclinical Studies

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ABSTRACT There is considerable preclinical evidence for a relationship between noradrenergic brain systems and behaviors associated with stress and anxiety. The majority of noradrenergic neurons are located in the locus coeruleus (pons), with projections throughout the cerebral cortex and multiple subcortical areas, including hippocampus, amygdala, thalamus, and hypothalamus. This neuroanatomical formation of the noradrenergic system makes it well suited to rapidly and globally modulate brain function in response to changes in the environment, as occurs during the presentation of stressors. Stress exposure is associated with an increase in firing of the locus coeruleus and with associated increased release and turnover of norepinephrine in brain regions which receive noradrenergic innervation. Increased firing of the locus coeruleus is also associated with behavioral manifestations of fear, such as arched back and piloerection in the cat. Exposure to chronic stress results in long-term alterations in locus coeruleus firing and norepinephrine release in target brain regions of the locus coeruleus. Norepinephrine is also involved in neural mechanisms such as sensitization and fear conditioning, which are associated with stress. These findings are relevant to an understanding of psychiatric disorders, such as panic disorder and post-traumatic stress disorder (PTSD), the symptoms of which have been hypothesized to be related to alterations in noradrenergic function. © 1996 Wiley-Liss, Inc.

INTRODUCTION

The relationship between noradrenergic function and states of anxiety and stress has long been a subject of interest. Anxiety is phenomenologically similar to states of fear, with the difference that fear is related to a real threat, while anxiety is an excessive response when little or no real danger is present. Anxiety can be conceptualized as an abnormality of the fear response. What is normally adaptive during situations of extreme physical threat, including increases in heart rate and blood pressure, increased vigilance, hyperarousal, exaggerated startle, and an enhancement of memory storage, are considered to be part of an anxiety response when little or no threat is present (Bremner and Charney, 1994). The locus coeruleus/noradrenergic system plays an important role in the fear response and anxiety. Other brain systems are also involved in the fear response and anxiety, including the corticotropin-releasing factor (CRF)/hypothalamic-pituitary-adrenal (HPA) axis system, benzodiazepine, dopamine, opiate, and serotonergic systems. These neuropeptidal and neurotransmitter systems function in a coordinated manner with norepinephrine as has been reviewed elsewhere (Bremner et al., 1993a; Charney et al., 1993; Chrousos and Gold, 1992). Specific brain regions involved in the stress response include amygdala, hippocampus, thalamus, hypothalamus, prefrontal cortex, central gray, and locus coeruleus. In this paper we review research findings from animal studies on the relationship between noradrenergic brain function and behaviors related to anxiety and stress. In a second paper, we will review clinical studies on the relationship between norepinephrine and anxiety and stress.

ANATOMY AND PHYSIOLOGY OF THE LOCUS COERULEUS/NORADRENERGIC SYSTEM

The majority of norepinephrine cell bodies in the brain are located in the locus coeruleus (dorsal pons), with some cell bodies also in the lateral tegmental area (reviewed in Holets, 1990). Ascending noradrenergic fibers of the locus coeruleus synapse throughout the cerebral cortex and in cerebellum, cingulate gyrus, thal-

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amus, hippocampus, hypothalamus, amygdala, bed nucleus of the stria terminalis, and nucleus accumbens, whereas descending fibers synapse at the level of the thoracic spinal cord (reviewed in Craig, 1992; Foote et al., 1983; Heal and Marsden, 1990; Morrison et al., 1978). In contrast to its broad efferent output, the locus coeruleus has a relatively restricted afferent input. Aston-Jones et al. (1986, 1991a), using wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP), have reported that major inputs to the locus coeruleus are the nucleus paragigantocellularis and the nucleus prepositus hypoglossi, both of which nuclei are located in the rostral medulla, as well as minor inputs from the paraventricular nucleus of the hypothalamus, midbrain peri-aqueductal gray, and the ventromedial pericoerulear region. Other investigations have found a broader range of afferent inputs to the locus coeruleus. One study using retrograde transport of nonconjugated horseradish peroxidase found evidence for innervation of the locus coeruleus from the central nucleus of the amygdala, insular cortex, preoptic area, lateral and dorsomedial hypothalamus, stria terminalis, central grey, nucleus tractus solitarius, reticular formation, and raphe (Cedarbaum and Aghajanian, 1978a,b). Anterograde labeling with *Phaseolus vulgaris* leucoagglutinin (PHA-L) in the cat and monkey has shown terminal fibers in the locus coeruleus following injections in lamina I cells (Craig, 1992). Electrophysiological studies have also found that spinal cord lamina I cells terminated in the locus coeruleus. Evidence against the hypothesis that the nucleus paragigantocellularis and the nucleus prepositus hypoglossi are the predominant efferents to the locus coeruleus is also not supported by the finding that destruction of these nuclei does not block locus coeruleus responses to somatosensory stimuli (Rasmussen and Aghajanian, 1989a,b). Discrepant observations related to afferent inputs to the locus coeruleus may be due to the fact that WGA-HRP is not an efficient retrograde marker for lamina I neurons (Craig et al., 1989).

The neuroanatomy of the afferent and efferent inputs to the locus coeruleus is suggestive of the role it may play in the stress response. The central nucleus of the amygdala, which is involved in the conditioned fear response, has efferent outputs to the lateral nucleus of the hypothalamus, which in turn mediates the increase in heart rate and blood pressure associated with fear. Projections of the lateral nucleus of the hypothalamus to the locus coeruleus (as well as descending projections of the locus coeruleus to brainstem areas which regulate cardiovascular function) could explain the association between firing of the locus coeruleus and increases in peripheral heart rate and blood pressure. The fact that the nucleus paragigantocellularis, a major input to the locus coeruleus, also controls peripheral sympathetic activity, has led to the hypothesis that this nucleus activates central and peripheral sympathetic systems in parallel (Aston-Jones et al., 1986, 1991a). Projections from sensory relay areas such as the nucleus tractus solitarius and the raphe to the locus coeruleus provide a potential explanation for how the locus coeruleus is responsive to physical sensations and changes in cardiovascular function, as occurs when the body is stressed by a sudden hypotensive crisis. The amygdala integrates sensory information from a variety of primary sensory areas during the stress response. Direct projections from the amygdala to the locus coeruleus could explain how primary sensory information related to fear-inducing stimuli activates the locus coeruleus. The broad range of afferent outputs of the locus coeruleus suggests that its function is to rapidly and globally modulate brain function during situations of stress (Olpe et al., 1985).

A variety of neurotransmitters and neuropeptides involved in the stress response regulate locus coeruleus function. The nucleus paragigantocellularis provides excitatory amino acid (Akaoka and Aston-Jones, 1991; Ennis and Aston-Jones, 1988; Rasmussen and Aghajanian, 1989a,b) adrenergic and noradrenergic inhibitory input to the locus coeruleus (Aghajanian et al., 1977; Cedarbaum and Aghajanian, 1976, 1977; reviewed in Aston-Jones et al., 1991a). Cells in the nucleus paragigantocellularis, nucleus prepositus hypoglossi, and paraventricular nucleus of the hypothalamus contain CRF, which is excitatory (Valentino et al., 1991), where endogenous opiates in the nucleus paragigantocellularis and nucleus prepositus hypoglossi are inhibitory to the locus coeruleus (Korf et al., 1974; reviewed in Aston-Jones et al., 1991a). The nucleus prepositus hypoglossi has primarily a GABAergic inhibitory input to the locus coeruleus (reviewed in Aston-Jones et al., 1991a). Benzodiazepines are inhibitory to the locus coeruleus (Grant et al., 1980), while serotonin (Akaoka and Aston-Jones, 1993; Aston-Jones et al., 1991c), attenuates responses of locus coeruleus neurons to excitatory amino acids. Other neurotransmitters which regulate the locus coeruleus include neurotensin, vasoactive intestinal peptide, and substance P (reviewed in Aston-Jones et al., 1991a).

EFFECTS OF NOREPINEPHRINE ON CENTRAL BRAIN FUNCTION

Norepinephrine has both inhibitory and excitatory effects on neuronal function, the effect being determined by the postsynaptic receptor upon which it is acting (Unnerstall et al., 1984). Studies using iontophoretic application have shown an inhibitory effect of norepinephrine acting through β-receptors (Egawa et al., 1988; Herrling, 1981) on spontaneous neuronal activity in rat hippocampus (Segal, 1981; Segal and Bloom, 1974a), rat cerebellum (Freedman et al., 1977; Hoffer et al., 1973), and monkey auditory cortex (Foote et al., 1975). Electrical stimulation of the locus coeruleus also results in an inhibition in hippocampus (Segal and

Bloom, 1974a,b) and cerebellum (Hoffer et al., 1973), whereas destruction of noradrenergic pathways has the opposite effect. A β-receptor–mediated decrease in background neuronal activity has led to the hypothesis that norepinephrine increases the signal-to-noise ratio of neuronal activity, which potentiates the ability of the organism to attend to relevant stimuli (Aston-Jones et al., 1991a,b; Madison and Nicoll, 1982; Woodward et al., 1979). Stimulation of alpha$_2$ receptors has also been shown to have an inhibitory effect on neurons of the cerebral cortex (Lomasney et al., 1991).

Norepinephrine acting through α-receptors in deep layers of cerebral cortex has been shown to have an excitatory effect on neuronal function (Bevan et al., 1977; Waterhouse et al., 1981). Application of norepinephrine to subcortically projecting neurons in layer V of the cerebral cortex results in an α_1-noradrenergic receptor-mediated shift in firing pattern from spontaneously bursting to single-spike activity. These neurons project to the superior colliculus and pons, and may be responsible for transitions from sleep to wakefulness and increased arousal, which is crucial in the stress response (Wang and McCormick, 1993). The thalamus acts as a gate for incoming sensory information which is relayed to cortical and subcortical areas (McCormick and Huguenard, 1992). A shift in neurons of the lateral geniculate nucleus of the thalamus from rhythmic burst firing to single spike firing is mediated by norepinephrine through α_1-noradrenergic receptors. This shift is functionally associated with an increase in responsiveness to excitatory potentials, which increases the transmission of sensory inputs (McCormick, 1992a,b), and also mediates the transition from sleep to wakefulness (McCormick and Pape, 1990; Steriade et al., 1993; Von Krosigk et al., 1993). The ability to rapidly shift from states of sleep or decreased consciousness to a state of high attentiveness to sensory information is of course highly adaptive in survival and therefore in the stress response. Alterations in noradrenergic function in patients with post-traumatic stress disorder (PTSD) and panic disorder could affect thalamocortical relay neurons, leading to an impairment of transmission of sensory information from the outside world. This could result in symptoms of dissociation, which are characterized by distortions of sensory and time perception, and which are associated with PTSD and panic disorder (Bremner et al., 1993a; Bremner et al., 1995a).

ACTIVATION OF THE LOCUS COERULEUS/ NORADRENERGIC SYSTEM WITH STRESS

Stress results in a rapid and robust activation of the locus coeruleus/noradrenergic system. During states of rest (Aghajanian et al., 1977; Aghajanian, 1978; Bunney et al., 1975), sleep (Foote et al., 1980), feeding and grooming (Aston-Jones, 1985; Grant and Redmond, 1984; Rasmussen et al., 1986), locus coeruleus neurons discharge in a slow, phasic manner. A variety of novel stimuli have been shown to activate the locus coeruleus in several animal species, including tail compression and pin-prick in the anesthetized rat (Korf et al., 1974; (Cedarbaum and Aghajanian, 1978b; Grant et al., 1980; Korf et al., 1974) pressure on the extremities, immobilization, light flashes and tones in the awake rat (Aston-Jones and Bloom, 1981a,b; Foote et al., 1980; Korf et al., 1974), clicks, flashes of light, and tail pinches in the awake cat (Rasmussen et al., 1986), and presentation of a preferred food, the appearance of an unfamiliar person (Foote et al., 1980; Grant and Redmond, 1984), pin prick, and restraint in the awake monkey (Aston-Jones, 1985; Grant and Redmond, 1984). These studies show that the locus coeruleus is activated by novel stimuli.

Behaviors which are characteristically seen during situations of stress and fear are associated with an increase in activation of the locus coeruleus/noradrenergic system. Infusion of norepinehprine into the hypothalamus of cats results in defensive/aggressive behaviors such as hissing, growling, and ear retraction (Barrett et al., 1987). An increase in firing from single-unit noradrenergic neurons in the locus coeruleus of cats is seen following exposure to noxious air puff stimuli (Rasmussen and Jacobs, 1986) or visual threat (Rasmussen et al., 1986). An increase in heart rate and blood pressure, which is a central part of the stress response, is associated with a parallel activation of the locus coeruleus and increases in plasma norepinephrine following both footshock in rats (Grant et al., 1980) and white noise and restraint stress in cats, with the increase in locus coeruleus activity closely coupled to the stress-induced acceleration of heart rate (Abercrombie and Jacobs, 1987a). An adaptation response which results in diminished heart rate activation in response to white noise is associated with parallel decreases in locus coeruleus and plasma norepinephrine activation (Abercrombie and Jacobs, 1987b). A two- to threefold increase in locus coeruleus activity in the cat associated with defensive behavios such as arched back, piloerection, flattened ears, increased heart rate and blood pressure, and mydriasis follows stressors such as seeing a dog or an aggressive cat (Levine et al., 1990). Behaviorally activating but non-stressful stimuli, such as seeing an inaccessible rat, do not result in activation of these neurons (Abercrombie and Jacobs, 1987a; Rasmussen et al., 1986). Electrical stimulation of the locus coeruleus in seat-restrained monkeys, which results in increased levels of norepinephrine in the brain, cerebrospinal fluid, and plasma, is associated with behaviors seen in the wild when the animal is threatened. If the animal is asleep, it will wake up and begin a series of behaviors which may include head and body turning, eye scanning, tongue movement, scratching, jerking, hand-wringing, escape struggling, and pulling hair and skin (Redmond, 1979a,b; Redmond and Huang, 1979; Redmond et al., 1976). In awake, seat-restrained monkeys, social signals seen during threat (mouth open,

TABLE I. Regional increases in norepinephrine turnover in the brain with stress[1]

Author	Stressor	Cortex	Hipp	Amyg	Hypo	Thal	LC	Pons	BG	Finding
Acute stressors										
Weiss et al. (1970)	Footshock	+								Dec. NE
Korf et al. (1973a)	Footshock	+								Dec. NE, Inc. MHPG
Tanaka et al. (1982)	Footshock	+		+		+		+		Dec. NE, Inc. MHPG
Anisman and Zacharko (1985)	Footshock	+	+							Dec. NE, Inc. MHPG
Tanaka et al. (1983)	Footshock		+							Dec. NE, Inc. MHPG
Tanaka et al. (1982)	Footshock				+					Increased MHPG
Stone (1975)	Footshock				+					Increased MHPG
Glavin et al. (1983)	Restraint		+	+	+					Decreased NE
Glavin et al. (1983)	Restraint		+	+	+	+			+	Increased MHPG
Shirao et al. (1988)	Restraint	+	+	+	+		+			Increased MHPG
Weiss et al. (1981)	Swim	+			+		+			Decreased NE
Irwin et al. (1986)	Shock (mice)		+		+					Dec. NE, Inc. MHPG
Yokoo et al. (1990)	Footshock				+					Inc. NE release
Abercrombie et al. (1988)	Footshock		+							Inc. NE release
Rossetti et al. (1990)	Footshock	+								Inc. NE release
Korf et al. (1973a,b)	Footshock						+			Inc. NE release
Petty et al. (1993)	Footshock		+							Inc. NE release
Abercrombie et al. (1988)	Restraint		+							Inc. NE release
Rossetti et al. (1990)	Restraint	+								Inc. NE release
Korf et al. (1973a,b)	Restraint						+			Inc. NE release
Tanaka (1991)	Restraint			+						Inc. NE release
Melia et al. (1992)	Footshock						+			Inc. TH prod.
Acute stressors after a period of exposure to chronic stress										
Weiss et al. (1970)	Footshock	+								Dec. NE
Weiss et al. (1981)	Footshock	+								Dec. NE
Adell et al. (1988)	Restraint				+			+		Dec. NE, Inc. MHPG
Nissenbaum et al. (1991)	Footshock		+							Inc. NE rel., MHPG release
Anisman and Zacharko (1985)	Footshock	+	+		+					Inc. NE release
Irwin et al. (1986)	Footshock	+								Inc. NE release
Anisman et al. (1985)	Footshock		+		+					Inc. MHPG
Cassens et al. (1980)	Footshock	+					+			Inc. MHPG
Stone et al. (1975)	Footshock	+					+			Inc. MHPG
Anisman and Zacharko (1985)	Footshock	+								Inc. MHPG
Irwin et al. (1986)	Shock (mice)		+		+					Inc. MHPG
Nissenbaum et al. (1991)	Footshock		+				+			Inc. TH prod.

[1]Dec. NE, decreased brain region content of norepinephrine; Inc. MHPG, increased brain region concentration of the norepinephrine metabolite MHPG; Inc. NE release, increased release of norepinephrine with stressor measured by microdialysis; Inc. TH prod., increased production of the rate-limiting enzyme of norepinephrine synthesis, tyrosine hydroxylase. Findings in stress of an increased release of norepinephrine, a decrease in brain norepinephrine concentration, increases in the norepinephrine metabolite MHPG, and increases in tyrosine hydroxylase are consistent with an increased synthesis, utilization, and metabolism of norepinephrine, with the increased synthesis not able to keep up with the increase in demand.

with stare), are associated with an increase in locus coeruleus activity to a greater degree than other stimuli such as flashes of light and tones (Grant and Redmond, 1984). These studies show that an increase in locus coeruleus activity/noradrenergic activity, with increased norepinephrine release in the brain, is associated with fear- and anxiety-related behaviors.

Pharmacological agents which affect locus coeruleus firing modulate behaviors associated with stress. Administration of the α_2 antagonists yohimbine and piperoxane, which results in an increase in firing of the locus coeruleus (Rasmussen and Jacobs, 1986) and increased release of norepinephrine in target brain structures, produces behaviors consistent with anxiety or fear in both rats and monkeys (Redmond, 1979a,b). Blockade of α_2 receptors with the α_2 antagonist idazoxan results in an increased responsiveness of the LC to excitatory stimuli (Simson and Weiss, 1989). Animals with a history of exposure to chronic stress have an increase in norepinephrine release in the hippocampus following administration of idazoxan, suggesting that chronic stress is associated with alterations in α_2 receptor function (Nissenbaum and Abercrombie, 1993). Agents which decrease firing in the locus coeruleus, including opiates, benzodiazepines (Drugan et al., 1984; Rasmussen and Jacobs, 1986; Tanaka et al., 1990), ethanol (Shirao et al., 1988), and clonidine (Aghajanian, 1978; Aghajanian and VanderMaelen, 1982; Redmond, 1979a,b), decrease anxiety behaviors. Prevention of the depletion of norepinephrine by preadministration of the α_2-noradrenergic receptor agonist clonidine blocks the development of learned helplessness following exposure to inescapable stress (Anisman et al., 1980).

Increases in locus coeruleus activity during stress are associated with an increase in regional turnover and release of norepinephrine in brain regions which are innervated by the locus coeruleus. As can be seen in Table I, multiple stressors, including footshock, restraint, and forced swim, result in increased turnover of norepinephrine in several target brain regions of the locus coeruleus, including cerebral cortex, hippocampus, hypothalamus, and amygdala. Increased turnover of norepinephrine is manifested by an increased release of norepinephrine in these brain regions as measured

by microdialysis, increased levels of the norepinephrine metabolite MHPG, decreased brain norepinephrine content (suggestive of increased utilization), and increased levels of the rate-limiting enzyme of norepinephrine synthesis, tyrosine hydroxylase. These studies support the hypothesis that the locus coeruleus/noradrenergic system is involved in the mediation of fear-related behavios and anxiety.

Stress-induced increases in norepinephrine also modulate gene transcription. The c-*fos* gene (together with c-*jun*) codes for a protein that serves to regulate the transcription of genes having the AP-1 consensus element. This gene is a member of the group of immediate early genes (IEGs) which have the ability to be activated by various neurotransmitters. Restraint stress increases c-*fos* immunoreactivity in brain regions which have been implicated in the stress response, such as prefrontal cortex (Deutch et al., 1991; Stone et al., 1993). This effect of restraint stress on c-*fos* appears to be related to stress-induced release of norepinephrine acting on postsynaptic β-receptors (Bing et al., 1992; Stone et al., 1992). The α_2 antagonist yohimbine, which increases brain norepinephrine release, increases c-*fos* in a manner similar to stress, while lesions of the locus coeruleus block this effect (Stone et al., 1993). These studies suggest that norepinephrine released during stress has global modulatory actions on gene transcription which could lead to rapid changes in cellular metabolism and other functions. This type of rapid effect on brain function could be critical during acute stress.

ROLE OF NOREPINEPHRINE IN THE NEURAL MECHANISMS OF SENSITIZATION, CONDITIONED FEAR, AND EXTINCTION

Preclinical studies of the effects of chronic stress on regional norepinephrine turnover illustrate the mechanism of stress sensitization. Stress sensitization, which is relevant to the neurobiology of psychiatric disorders related to stress and anxiety such as panic disorder and PTSD, refers to the increase in responsiveness which occurs with reexposure to a stressor in organisms which have had a history of previous exposure to that specific stimulus. Illustrating this concept, animals with a prior history of exposure to chronic stress show an increase in norepinephrine release, 3-methyoxy-4-hydroxyphenylglycol (MHPG), and tyrosine hydroxylase upon reexposure to an acute stimulus, for several brain regions outlined in Table I, including hippocampus, hypothalamus, and cortex. When synthesis is not able to keep up with demand, chronic stress results in a decrease in brain norepinephrine content with associated behavioral changes which have been termed learned helplessness (Petty et al., 1993; Weiss et al., 1970, 1981). Chronic stress also results in an increased firing of the locus coeruleus (Korf et al., 1973a,b; Pavcovich et al., 1990; Simson and Weiss, 1988a,b). Animals exposed to chronic inescapable shock which is associated with learned helplessness have been shown to have an increase in responsiveness of the locus coeruleus to an excitatory stimulus in comparison to animals exposed to escapable shock. The authors hypothesized that this increase in LC responsiveness may be due to a depletion of NE and a subnormal activation of α_2-noradrenergic receptors at the level of the LC (Simson and Weiss, 1988a,b). Consistent with this idea, a decrease in density of α_2-noradrenergic receptors specific to the hippocampus and amygdala, with an increase in affinity in the amygdala, has been associated with acute cold-restraint stress in the rat (Nukina et al., 1987). In addition, chronic, but not acute, stress in rats blocks the reduction of locomotor activity normally associated with administration of the α_2 agonist clonidine, suggesting a decreased responsiveness of α_2 receptors following chronic stress (Cancela et al., 1988). An increased locus coeruleus responsiveness and regional norepinephrine release with re-exposure to stressors in individuals with a prior history of stress exposure may explain why patients with panic disorder and PTSD have an increase in sensitivity to stressors compared to individuals without psychiatric disorders.

Clinical studies demonstrate that stress sensitization is a mechanism which is seen in patients with PTSD. In a study of Israeli combat veterans exposed to the recurrent stressors of participation in two successive wars, soldiers with a history of acute combat stress reaction (ACSR) following the original conflict were more likely to develop ACSR after participation in the subsequent conflict (Solomon et al., 1987). In addition, Vietnam combat veterans with combat-related post-traumatic stress disorder have been shown to have an increased rate of childhood physical abuse in comparison to Vietnam combat veterans without a history of post-traumatic stress disorder (Bremner et al., 1993b). This suggests that early life stress may sensitize the individual to the development of stress-related psychopathology with exposure to subsequent stressors.

Norepinephrine is also involved in the neural mechanism of conditioned fear (Davis, 1986). Conditioned fear refers to the development of fear responses in response to a previously neutral stimulus which has been paired with a fear-inducing stimulus. A normally neutral stimulus (or something which typically has no effect on the animal, such as a bright light), is paired with an aversive stimulus such as electric shock. With repetitive pairing of the light and the shock, a learning process occurs (conditioning) in which the light alone eventually causes an increase in fear responses. The shock in this example is termed the unconditioned stimulus, because no training was required for it to induce fear, while the light is referred to as the conditioned stimulus, as the training trials pairing it with the shock were required for it to develop the capacity to induce fear-related responses. Conditioned fear is often tested in the laboratory using the fear potentiated startle paradigm, where

with repetitive pairing of a light and shock there is a potentiation of the startle response with the light alone (Davis, 1992). Stress results in an increased release of norepinephrine in the amygdala, which modulates conditioned fear responsiveness. Consistent with this, norepinephrine depletion in rats has been shown to result in an impairment of fear responding to explicit cues, and an enhancement of responding to contextual cues (Selden et al., 1990). The amygdala, through reciprocal connections with the locus coeruleus, also may modulate locus coeruleus firing. For instance, in the awake cat, an increase in locus coeruleus firing follows exposure to a stimulus which was previously paired with a noxious air puff, whereas stimuli paired with a food reward does not result in an increase in locus coeruleus firing (Rasmussen and Jacobs, 1986).

Pathways from the amygdala to the lateral hypothalamus also effect peripheral sympathetic responses to stress (Iwata et al., 1986; Sawchenko and Swanson, 1981). Electrical stimulation of the amygdala in cats results in peripheral signs of autonomic hyperactivity and fear-related behaviors seen in the wild when the animal is being attacked or is attacking, including alerting, chewing, salivation, piloerection, turning, facial twitching, arching of the back, hissing, and snarling (Gunne and Reis, 1963; Hilton and Zbrozyna, 1963), which is associated with a depletion of norepinephrine in the brain and epinephrine and norepinephrine in the adrenal, suggesting an increase in catecholamine turnover (Gunne and Reis, 1963). Electrical stimulation of the amygdala in human subjects results in an increase in heart rate and blood pressure, increased muscle tension, and subjective sensations of fear or anxiety (Chapman et al., 1954). These findings suggest that norepinephrine may be acting through the central nucleus of the amygdala and its efferent outputs to activate conditioned fear responses, fear-related behaviors, and peripheral sympathetic responses associated with stress.

The hippocampus also plays an important role in conditioned fear related to the context of a fear-inducing situation. In conditioned fear response experiments where a tone (conditioned stimulus) is paired with electric footshock (unconditioned stimulus), re-exposure of the animal to the tone will result in conditioned fear responses (increase in "freezing" responses, which is characteristic of fear), even in the absence of the shock. In addition, reintroduction to the context of the shock, or the environment where the shock took place (i.e., the testing box), even in the absence of the shock or the tone, will result in conditioned fear responses. Lesions of the amygdala before fear conditioning block fear responses to both simple stimuli (tone) and to the context of the footshock. Lesions of the hippocampus, on the other hand, do not interfere with acquisition of conditioned emotional responses to the tone in the absence of the shock, although they do interfere with acquisition of conditioned emotional responses to the context (Phillips and LeDoux, 1992). Lesions of the hippocampus 1 day after fear conditioning (but not as much as 28 days after fear conditioning) also abolish context-related fear responses, but not fear related to the cue (tone), whereas lesions of the amygdala block fear responses to both the cue and the context (Kim and Fanselow, 1992). These studies suggest that the hippocampus has a time-limited role in fear responses to complex phenomena with stimuli from multiple sensory modalities, but not to stimuli from simple sensory stimuli. The amygdala integrates information from multiple sensory modalities and effects the conditiond emotional response. The role of the hippocampus is to formulate conditioned responses to complex spatially related stimuli. It probably does this by integrating complex spatially related stimuli and passing this information through the subiculum to the amygdala, which effects the stress response. Norepinephrine is involved in conditioned fear responses to complex spatial stimuli which are mediated by the hippocampus. Re-exposure of animals with a history of prior footshock (unconditioned stimulus) to the environment where footshock originally occurred (in this example, the conditioned stimulus) without footshock results in an increased release of norepinephrine within the hypothalamus (Yokoo et al., 1990). Re-exposure to the environment where shock occurred without re-exposure to shock also results in an increase in the norepinephrine metabolite MHPG in whole brain samples with associated fear-related behaviors such as increased defecation (Cassens et al., 1980). These findings suggest that norepinephrine may be a chemical substrate for the conditiond fear responsiveness to complex spatial stimuli which is mediated by the hippocampus.

Noradrenergic contributions to conditioned fear are relevant to panic disorder and PTSD. For instance, exposure to a fear-inducing situation in a patient with panic disorder, such as a bridge or a crowded shopping mall, may be associated with an increased release of norepinephrine in specific brain regions such as the amygdala, which in turn stimulates a panic attack. In PTSD patients the sound of a car backfiring (a conditioned stimulus associated with the stress of combat) may be associated with an increase in norepinephrine release in some of the specific brain regions outlined above, such as amygdala and hippocampus, which mediate conditioned fear responses to simple and complex stimuli, respectively. For a victim of rape, being in the dark, in a deserted alleyway, a cold winter evening, and other details which remind the victim of the original rape may be enough to elicit a conditioned fear response. Norepinephrine release in the amygdala may modulate the simple sensory aspects of this experience (darkness, feelings of cold), while norepinephrine release in the hippocampus affects the integration of all of the aspects of the experience, which lead to a recall of the exact event, and a conditioned fear response to the sum of the

combined stimuli. Patients with PTSD recall traumatic events in a different way than normal memories. Norepinephrine release in the amygdala and hippocampus may play a role in the mechanism of traumatic recall.

Noradrenergic brain systems may also be involved in a failure of extinction which is associated with stress exposure. In the example used above, repetitive pairing of a light (conditioned stimulus) and a shock (unconditioned stimulus) will result in a conditioned fear response to the light alone. The neural mechanism of extinction is illustrated by the fact that repeated exposure to the light alone will eventually lead to the loss of conditioned responding. Studies have shown that this extinction is due in fact to an inhibition by cortical areas of subcortical brain structures (such as the amygdala) which mediate conditioned fear responding. A failure of extinction of conditioned emotional responding is a characteristic of patients with both PTSD and panic disorder. For example, a veteran who has a conditioned fear response of becoming startled and agitated with the sound of a car backfiring, which is associated with the original aversive stimulus of gunfire in Vietnam, does not become less agitated with repeated exposures to cars backfiring. In a parallel fashion, some patients with phobic anxiety may not have a diminution of conditioned emotional responses with repeated exposure to the phobic stimulus. One might speculate that a mechanism such as enhanced release of norepinephrine in hippocampus, amygdala, or other brain regions involved in stress and memory in patients with PTSD or panic disorder upon exposure to the fear-eliciting stimulus could play a role in the failure of extinction which is chaacteristic of these patients.

FUNCTIONAL NEUROANATOMICAL CORRELATES OF THE RELATIONSHIP BETWEEN NORADRENERGIC BRAIN SYSTEMS AND MEMORY

Recently the relationship between stress and memory has been a topic of increased focus. Accumulated preclinical evidence has shown that stress impairs memory function and leads to long-term alterations in brain regions involved in memory (Bremner et al., 1993c; Bremner et al., 1995b). Patients with PTSD have been shown to have deficits in short-term verbal memory (Bremner et al., 1993c), which is associated with a decrease in right hippocampal volume measured with magnetic resonance imaging (Bremner et al., 1995b). Memory function is also of interest in patients with panic disorder, as impairments in cognition are an important aspect of the disability associated with this disorder.

There has long been an interest in the relationship between noradrenergic function and memory (Sara, 1985a). The locus coeruleus projects to several brain areas which play a role in both memory function and the stress response. These regions include hippocampus and adjacent cortex, amygdala, thalamus, prefrontal cortex, parietal cortex, and cingulate cortex. Release of norepinephrine in these areas can modulate neuronal activity and gene transcription, and therefore the laying down of memory traces.

Early neuroanatomical studies showed that removal of the cerebral cortex of the cat, so that remaining brain regions included amygdala, thalamus, hippocampus, and hypothalamus, resulted in accentuated fearful responses to potentially threatening or novel stimuli, accompanied by signs of diffuse sympathetic activation such as increased blood pressure, sweating, piloerection, and increased secretion of epinephrine from the adrenal medulla (Cannon, 1931). This behavioral response become termed as "sham rage," and led to the original hypothesis that subcortical brain structures above the level of the midbrain, such as the hypothalamus, hippocampus, cingulate, entorhinal cortex, and thalamus, may be involved in emotional responses such as fear (Kluver and Bucy, 1937, 1939; Papez, 1937; reviewed in LeDoux, 1977). Maclean (1966) later added the amygdala to the "Papez Circuit" of "limbic" brain structures, so called because of their relationship to olfaction in evolution, which were hypothesized to play a role in fear and anxiety. These early neuroanatomical investigations were valuable in showing that brain regions involved in memory also play an important role both in fear-related behaviors seen naturally in the wild, and in manifestations of increased catecholaminergic activity.

Norepinephrine appears to play a role in modulating the acquisition and retention of memory traces. Lesions of the dorsal noradrenergic bundle, with fibers which project from the locus coeruleus to the cortex, result in an impairment in the acquisition of new information (reviewed in Robbins et al., 1985). Dorsal noradrenergic bundle lesions have been shown to result in an impairment in visual discrimination tasks when a distracting noise is presented immediately before the test, suggesting a specific role of norepinephrine in the attentional component of memory storage (Cole and Robbins, 1992). Toxin-induced catecholamine depletion of the principal sulcus region of the prefrontal cortex of the monkey causes deficits in delayed-response tasks, a test of working memory (Brozoski et al., 1979). Aging in the primate is associated with depletion of neuronal cell bodies in the locus coeruleus as well as a loss of norepinephrine terminals in target brain regions, including the prefrontal cortex, with associated deficits in working memory (Arnsten and Goldman-Rakic, 1985). Deficits in noradrenergic systems have been shown in clinical disorders which are associated with memory impairment, including Korsakoff's amnesia (Mair and McEntee, 1983) and Alzheimer's disease (Adolfsson et al., 1979).

Activation of the noradrenergic system, on the other hand, has been shown to enhance memory function. For

example, norepinephrine increases neuronal firing in the hippocampus, suggesting a possible role in the enhancement of memory storage (Madison and Nicoll, 1982). Other neurotransmitters and neuropeptides, including benzodiazepines, glucocorticoids, cholecystokinin, neuropeptide Y, opiate antagonists, and acetylcholine, some of which are modulated by norepinephrine, are involved in memory encoding and retrieval (McGaugh, 1989). Electrical stimulation of the locus coeruleus improves acquisition of information (Velly et al., 1985). In rats who had undergone a series of maze training trials, administration of the α_2 antagonist yohimbine, which increases norepinephrine release in the hippocampus and other target brain structures of the locus coeruleus, 25 days after the last training trial resulted in fewer errors in the delayed maze escape test upon retesting in comparison to placebo (Goldberg and Robertson, 1983). Administration of amphetamine, which increases both endogenous norepinephrine and dopamine release, also resulted in a significant reduction in errors in comparison to placebo. These findings are consistent with a facilitating effect of norepinephrine on memory retrieval (Sara, 1985b). Since rats use contextual cues, such as shadows on the side of the maze, in maze escape behaviors, this has led to the formulation that the locus coeruleus/norepinephrine system may be involved in modulating the response of hippocampal neurons to contextual cues.

Understanding the relationship between stress-induced activation of the noradrenergic system and memory function is relevant to the clinical treatment of patients with PTSD and panic disorder (Bremner and Charney, 1993). Patients have abnormal recall of trauma-related events which are characterized by intense emotional responding, increases in heart rate and blood pressure, and dissociative reliving of the experience through "flashbacks." Based on preclinical findings one can construct a model whereby potentiated release of norepinephrine in brain regions involved in memory following exposure to trauma-related environmental cues, combined with pathology at the level of these brain regions or in other neurotransmitter or neuropeptide systems, leads to traumatic recall.

COMMENT

Evidence from preclinical studies supports a role for the locus coeruleus/noradrenergic system in stress and anxiety. Much of this research involves studies of inescapable stress, which has been used as an animal model for both panic disorder and PTSD. The locus coeruleus/noradrenergic system is situated to be capable of rapidly modulating brain function in response to sudden changes in the environment, which is crucial in the stress response. Several lines of evidence from preclinical studies are consistent witha role for norepinephrine in anxiety and stress. Stress results in an increased synthesis, release, and turnover of norepinephrine in several brain regions which are involved in the stress response, including the cerebral cortex, hippocampus, hypothalamus, amygdala, and locus coeruleus. Behaviors related to fear, such as a cat seeing a dog, but not arousing, non-fearful situations, such as a cat seeing an inaccessible rat, are associated with an increase in firing of the locus coeruleus. Medications which decrease firing of the locus coeruleus result in a decrease in anxiety-type behaviors, whereas medications which increase firing of the locus coeruleus result in an increase in anxiety behaviors. These studies are consistent with an increase in activity of the locus coeruleus/norepinephrine system in stress and anxiety.

Noradrenergic brain systems are involved in the neural mechanisms of fear conditioning, sensitization, and extinction, which are relevant to animal models of stress and clinical conditions of panic disorder and PTSD. Fear conditioning refers to the phenomenon where an animal, after being exposed to a neutral stimuli and a stressful stimulus together, such as a light and an electric shock, will have fear responses to the light alone. Fear conditioning may be modulated by release of norepinephrine in brain structures which modulate this phenomenon, including the hippocampus and amygdala. In sensitization, previous exposure to a stressful stimulus will result in an increased responsiveness upon re-exposure to a secondary stressful stimulus. Re-exposure of an animal with a history of chronic stress results in a potentiated release of norepinephrine in the hippocampus. This may represent a mechanism whereby exposure to stressful stimuli in the environment is associated with recall of abnormal traumatic memories. Extinction is the loss of conditioned responding over time, which involves cortical inhibition of subcortical brain structures such as the amygdala. Noradrenergic brain systems may also be involved in a failure of extinction seen with exposure to chronic stress.

Animal models have been used extensively in the evaluation of the relationship between stress and anxiety. This work has provided convincing evidence that alterations in noradrenergic function are associated with behaviors which are seen in stress and anxiety. In a follow-up report we address the question of whether norepinephrine mediates behaviors related to stress and anxiety in human subjects, and whether alterations in noradrenergic function underlie the symptomatology of patients with psychiatric disorders of anxiety and stress, such as panic disorder and post-traumatic stress disorder (PTSD).

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Noradrenergic Mechanisms in Stress and Anxiety: II. Clinical Studies

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KEY WORDS Anxiety, Noradrenergic function, Posttraumatic stress disorder, Panic disorder, Generalized anxiety disorder, Obsessive-compulsive disorder

ABSTRACT Studies in animals have shown a relationship between alterations in noradrenergic brain system function and behaviors of anxiety and fear. These findings have generated the hypothesis that the symptoms seen in patients with anxiety disorders may be related to alterations in noradrenergic function. A number of clinical studies have tested this hypothesis, utilizing measures of catecholaminergic function such as heart rate and blood pressure, measurement of norepinephrine and its metabolites in urine and plasma and adrenergic receptor binding in platelets, as well as pharmacological challenge to the noradrenergic system. Acute stressors, such as public speaking, have been associated with an increase in heart rate, blood pressure, and norepinephrine and its metabolites in urine and plasma. Findings in patients with panic disorder at baseline related to heart rate, blood pressure, baseline norepinephrine and its metabolites, and platelet adrenergic receptors have been mixed, while the most consistent findings have been blunted growth hormone response to clonidine and increased 3-methoxy-4-hydroxy-phenylethylene-glucol (MHPG) and anxiety following stimulation of the noradrenergic system with yohimbine. Baseline measures of noradrenergic function in patients with posttraumatic stress disorder (PTSD) have also been mixed, while an increased heart, blood pressure and norepinephrine response to traumatic reminders, as well as increased behavioral (as well as different brain metabolic) response to yohimbine, have been found in PTSD. There are fewer studies of noradrenergic function in the other anxiety disorders, and the findings there have not been consistent. These studies provide evidence for increased noradrenergic responsiveness in panic disorder and PTSD, although there does not appear to be an alteration in baseline noradrenergic function in these patients.
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INTRODUCTION

Considerable interest has been focused on the relationship between norepinephrine and anxiety. We have reviewed in a previous paper the evidence from animal studies for a relationship between norepinephrine and behaviors related to anxiety and stress. In this current paper we review evidence from clinical studies which have examined the relationship between norepinephrine and behaviors of anxiety and fear in human subjects, as well as alterations in noradrenergic function in patients with psychiatric disorders related to anxiety and stress, panic disorder, and posttraumatic stress disorder (PTSD).

The interest in the relationship between norepinephrine and anxiety and stress is partially related to the observation that there is a similarity between behavioral states associated with increases in catecholaminergic function and those associated with anxiety and fear. For instance, one of the authors treated a female patient with panic disorder who had panic attacks about once a week, characterized by the sudden onset of an increase in heart rate, with flushing and trembling, sweating, dry mouth, and dizziness. She had the feeling that she could not swallow, and became restless and short of breath. Thoughts began rushing into her head, like "this is the big one," "I'm going to die of a heart attack." This was followed by a further increase in heart rate and blood pressure. At this point the patient typically lost complete control and had an overwhelming panic attack, which she described as one of the worst things that could happen to her, something that she

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