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Stress Impairs α_{1A} Adrenoceptor-Mediated Noradrenergic Facilitation of GABAergic Transmission in the Basolateral Amygdala

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Intense or chronic stress can produce pathophysiological alterations in the systems involved in the stress response. The amygdala is a key component of the brain's neuronal network that processes and assigns emotional value to life's experiences, consolidates the memory of emotionally significant events, and organizes the behavioral response to these events. Clinical evidence indicates that certain stress-related affective disorders are associated with changes in the amygdala's excitability, implicating a possible dysfunction of the GABAergic system. An important modulator of the GABAergic synaptic transmission, and one that is also central to the stress response is norepinephrine (NE). In the present study, we examined the hypothesis that stress impairs the noradrenergic modulation of GABAergic transmission in the basolateral amygdala (BLA). In control rats, NE (10 μM) facilitated spontaneous, evoked, and miniature IPSCs in the presence of β and α_2 adrenoceptor antagonists. The effects of NE were not blocked by α_{1D} and α_{1B} adrenoceptor antagonists, and were mimicked by the α_{1A} agonist, A61603 (1 μM). In restrain/tail-shock stressed rats, NE or A61603 had no significant effects on GABAergic transmission. Thus, in the BLA, NE acting via presynaptic α_{1A} adrenoceptors facilitates GABAergic inhibition, and this effect is severely impaired by stress. This is the first direct evidence of stress-induced impairment in the modulation of GABAergic synaptic transmission. The present findings provide an insight into possible mechanisms underlying the antiepileptogenic effects of NE in temporal lobe epilepsy, the hyperexcitability and hyper-responsiveness of the amygdala in certain stress-related affective disorders, and the stress-induced exacerbation of seizure activity in epileptic patients.

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INTRODUCTION

Many components of the biological response to emotional stressors are of vital importance in enabling the individual to cope with stress. However, it is well known that excessive or repeated stress can have detrimental effects on health that are often associated with functional alterations in the systems involved in the stress response (Vermetten and Bremner, 2002; Vanitallie, 2002; McEwen, 2002; Pawlak *et al*, 2003). The amygdala is a key component of the brain's neuronal network that determines the emotional significance of external events (LeDoux, 1992; Davis, 1994; Breiter *et al*, 1996; Schneider *et al*, 1997; LaBar *et al*, 1998; Buchel *et al*, 1998; Whalen *et al*, 1998; Baird *et al*, 1999; Davidson *et al*, 1999; Davidson and Slagter, 2000; Buchel and Dolan, 2000). Via efferent pathways to the hypothalamus, the amygdala can also trigger the neuroendocrine cascades that are part of the stress response (Habib *et al*, 2001; Pitkänen, 2000; Davis, 1992) and via reciprocal connections with the cerebral cortex and limbic structures, it modulates the orchestration of the behavioral response (Goldstein *et al*, 1996; Pitkanen *et al*, 2000). Therefore, understanding the changes in the amygdala's physiology and function induced by stress is critical in understanding the pathophysiology of stress, and may aid the development of new therapeutic strategies for the prevention and treatment of stress-related, affective disorders.

Different lines of evidence point to the possibility that the function of the GABAergic system may be impaired by stress. First, in a number of brain regions, benzodiazepine receptor binding is altered by stress (Lippa *et al*, 1978; Medina *et al*, 1983; Miller *et al*, 1987; Weizman *et al*, 1989; Bremner *et al*, 2000). Second, in certain stress-related psychiatric disorders, the amygdala exhibits higher than normal levels of basal activity (Abercrombie *et al*, 1998; Drevets, 1999), or exaggerated responses to fearful stimuli (Rauch *et al*, 2000; Villarreal and King, 2001). Since the GABAergic system is a primary regulator of neuronal excitability, pathophysiological changes in GABAergic transmission may underlie the amygdala's hyper-responsiveness and hyperexcitability in these emotional disorders.

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Third, many psychotropic drugs that are effective in the treatment of emotional disorders target or influence GABAergic transmission. Fourth, stress exacerbates the frequency of seizures in epileptic patients (Temkin and Davis, 1984; Frucht *et al*, 2000). However, there is no direct evidence, so far, for stress-induced impairment in GABAergic synaptic transmission.

One of the modulators of GABA release is norepinephrine (NE), which is also central to the stress response. During stress, there is a dramatic increase in noradrenergic activity following the peripheral release of epinephrine from the adrenal glands, and the central release of NE, predominantly from the locus ceruleus (Stanford, 1995; Bremner *et al*, 1996). The amygdala receives dense noradrenergic afferents from the locus ceruleus (Pitkänen, 2000), as well as from other brain regions such as the nucleus of the solitary tract (Pitkänen, 2000; Clayton and Williams, 2000; Williams *et al*, 2000). During stress, there is a strong enhancement of NE release in the amygdala (Galvez *et al*, 1996; Stanford, 1995; Quirarte *et al*, 1998; Tanaka *et al*, 2000). The short- and long-term consequences of stress-induced excessive NE release on amygdala's physiology are unknown.

NE modulates GABAergic inhibition primarily via the α_1 subtype of adrenergic receptors (Gellman and Aghajanian, 1993; Alreja and Liu, 1996; Bergles *et al*, 1996; Kawaguchi and Shindou, 1998). There is evidence suggesting that α_1 adrenoceptors are affected by stress. Thus, chronic stress, in rats, reduces the expression of these receptors in the hypothalamus and brain stem (Miyahara *et al*, 1999). α_1 adrenoceptor binding is also reduced in depressed patients (Crow *et al*, 1984; Gross-Isseroff *et al*, 1990), and blockade of these receptors in rats increases depressive behavior (Stone and Quartermain, 1999). The physiological implications of stress-induced reduction in α_1 adrenoceptor activity are not known.

In the present study, we investigated whether NE modulates GABAergic transmission in the basolateral nucleus of the amygdala (BLA), and if so, whether the noradrenergic modulation of the GABAergic transmission is altered by exposure to stress. We studied the BLA because this amygdala region is heavily involved in the processing of emotional experiences, as it receives both direct and indirect thalamic and cortical inputs and is extensively interconnected with the prefrontal/frontal cortex and the hippocampus (Pitkänen, 2000). Furthermore, it appears that the BLA selectively (among the different amygdala nuclei) modulates the consolidation of emotional memories (Cahill and McGaugh, 1998; Ferry *et al*, 1999). Our results show that NE facilitates spontaneous, evoked, and action potential-independent, quantal GABA release in the BLA via the α_{1A} subtype of adrenergic receptors, and that exposure to stress severely impairs this α_1 adrenoceptor-mediated facilitation of GABA release.

METHODS

Animals and Stress Protocol

All animal experiments were performed in accordance with our institutional guidelines after obtaining the approval of the Institutional Animal Care and Use Committee (IACUC). Male, Sprague–Dawley rat pups were received with their mother at postnatal day (PND) 17, and housed in a climate-controlled environment on a 12 h light/dark cycle (lights on at 0700). On PND 21, the rats were weaned, assigned numbers, and randomly divided into control and stressed groups. They were housed individually, with food and water supplied *ad libitum*. The 'stressed group' was exposed to stress on PND 22, 23, and 24. The rats were killed and brain slices were prepared on PND 24 and 25. The experiments were performed in a blind manner. The investigators did not know whether they used a control or a stressed rat until the data were analyzed.

Stress exposure consisted of a 2-h per day session of immobilization and tail-shocks, for 3 consecutives days. The animals were stressed in the morning (between 0800 and 1200). They were restrained in a plexiglas tube, and 40 electric shocks (2 mA, 3 s duration) were applied at varying intervals (140–180 s). This stress protocol was adapted from the 'learned helplessness' paradigm in which animals undergo an aversive experience under conditions in which they cannot perform any adaptive response (Seligman and Maier, 1967; Seligman and Beagley, 1975). We stressed the rats for 3 consecutive days because it has been previously demonstrated that repeated stress sessions for 3 days is more effective than a single stress session in producing physiological and behavioral abnormalities, such as elevations in the basal plasma corticosterone levels, exaggerated acoustic startle responses, and reduced body weight (Servatius *et al*, 1995; Ottenweller *et al*, 1989). More stress sessions, beyond the 3 days, do not appear to produce greater physiological and behavioral changes (Servatius *et al*, 1995; Ottenweller *et al*, 1989).

Slice Preparation

Experimental procedures. The amygdala slice preparation has been described previously (Li *et al*, 2001). Briefly, the rats were anesthetized with halothane and then decapitated. The brain was rapidly removed and placed in an ice-cold artificial cerebrospinal fluid (ACSF) composed of (in mM) 125 NaCl, 2.5 KCl, 2.0 $CaCl_2$, 1.0 $MgCl_2$, 25 $NaHCO_3$, 1.25 NaH_2PO_4, and 11 glucose, bubbled with 95% O_2/5% CO_2. A block containing the amygdala region was prepared by rostral and caudal coronal cuts, and coronal slices, 400 μm thick, were cut using a Vibratome (series 1000, Technical Products International, St Louis, Missouri). Slices were kept in a holding chamber containing oxygenated ACSF at room temperature, and experiments started $\geqslant$1 h after slice preparation.

Electrophysiology

For whole-cell recordings, slices were transferred to a submersion-type recording chamber where they were continuously perfused with oxygenated ACSF at a rate of 4 ml/min. All experiments were carried out at 32°C. Tight-seal (>1 GΩ) whole-cell recordings were obtained from the cell body of neurons in the BLA region. Patch electrodes were fabricated from borosilicate glass and had a resistance of 1.5–5.0 MΩ when filled with a solution containing (in mM) Cs-gluconate, 135; $MgCl_2$, 10; $CaCl_2$, 0.1; EGTA, 1; HEPES, 10; QX-314, 20; NaATP, 2; Na_3GTP, 0.2 and Lucifer yellow, 0.4% (pH 7.3, 285–290 mOsm). Neurons were

visualized with an upright microscope (Nikon Eclipse E600fn) using the Nomarski-type differential interference optics through a $\times 60$ water immersion objective. Neurons with a pyramidal appearance were selected for recordings. During whole-cell recordings, neurons were filled passively with 0.4% Lucifer yellow (Molecular Probes, Eugene, Oregon) for *post hoc* morphological identification, as described previously (Braga *et al*, 2003). The fluorescence image of the dye-filled neurons was captured by a Leica DM RXA fluorescence microscope equipped with an SPOT2 digital camera and a laser scanning confocal microscope (Bio RAD, MRC-600). Neurons were voltage clamped using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Inhibitory postsynaptic currents (IPSCs) were pharmacologically isolated and recorded at a holding potential of −70 mV. Synaptic responses were evoked with sharpened tungsten bipolar stimulating electrodes (2 μm diameter, World Precision Instruments, Sarasota, Florida) placed in the BLA, 50–100 μm from the recording electrode. Stimulation was applied, at 0.1 Hz, using a photoelectric stimulus isolation unit having a constant current output (PSIU6, Grass Instrument Co., W. Warwick, RI). Access resistance (8–26 MΩ) was regularly monitored during recordings, and cells were rejected if it changed by more than 15% during the experiment. The signals were filtered at 2 kHz, digitized (Digidata 1322A, Axon Instruments, Inc.), and stored on a computer using the pCLAMP8 software (Axon Instruments, Inc.). The peak amplitude, 10–90% rise time, and the decay time constant of IPSCs were analyzed off-line using pCLAMP8 software (Axon Instruments) and the Mini Analysis Program (Synaptosoft, Inc., Leonia, NJ). Miniature IPSCs (mIPSCs) were analyzed off-line using the Mini Analysis Program (Synaptosoft, Inc., Leonia, NJ), and detected by manually setting the threshold for each mIPSC after visual inspection.

For field potential recordings, slices were transferred to an interface-type recording chamber maintained at 32°C, where they were perfused with ACSF at 0.7–1 ml/min. Field potentials were recorded in the BLA, while stimulation was applied to the external capsule, at 0.05 Hz (Aroniadou-Anderjaska *et al*, 2001). Recording glass pipettes were filled with 2 N NaCl (2–5 MΩ). Bipolar stimulating electrodes were constructed from twisted, stainless-steel wires, 50 μm in diameter. The field potentials were filtered at 1 kHz, and digitized on-line at 5 kHz.

All data are presented as mean ± SEM. For body weight data, sample size *n* refers to the number of rats. For electrophysiological experiments, sample size *n* refers to the number of slices. This corresponds to the number of neurons, in whole-cell recordings, as a single neuron was studied from each slice. From each rat, two slices were used for each type of experiment (whole-cell recordings or field potential recordings). The results were tested for statistical significance using the Student's paired *t*-test.

Drugs

The following drugs were used: D-(−)-2-amino-5-phosphonopentanoic acid (D-AP5, Tocris Cookson, Ballwin, Missouri), an NMDA receptor antagonist; 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, Tocris Cookson, Ballwin, Missouri), a potent AMPA/kainate receptor antagonist; (2*S*)-(+)-5,5-dimethyl-2-morpholineacetic acid (SCH50911, Tocris Cookson, Ballwin, Missouri), a $GABA_B$ receptor antagonist; bicuculline methiodide (Sigma), a $GABA_A$ receptor antagonist; tetrodotoxin (TTX, Sigma), a sodium channel blocker; DL-propranolol (Sigma), a β adrenoceptor antagonist; (1-[4-amono-6,7-dimethoxy-2-quinazolinyl]-4-[2-furanylcarbonyl]-piperazine hydrochloride (prazosin, Sigma), an α_1 adrenoceptor antagonist; yohimbine hydrochloride (Sigma), an α_2 adrenoceptor antagonist; *N*-[5-(4,5-dihydro-1H-imidazol-2-yl)-2-hydroxy-5,6,7,8-tetrahydro-naphthalen-1-yl]methanesulfonamide hydrobromide (A61603, Tocris Cookson, Ballwin, Missouri), a selective α_{1A} agonist (Knepper *et al*, 1995); chloroethylclonidine (CEC, Sigma), an irreversible antagonist that blocks both α_{1B} and α_{1D} adrenoceptors (Xiao and Jeffries, 1998); 8-[2-[4-(2-methoxyphenyl)-1-piperazinil]ethyl]-8-azaspiro[4,5]-decane-7,9-dione dihydrochloride (BMY 7378, Tocris Cookson, Ballwin, Missouri), a selective antagonist of α_{1D} adrenoceptors (Deng *et al*, 1996; Saussy Jr *et al*, 1996); 2-(2,6-dimethoxyphenoxyethy)aminomethyl-1,4-benzodioxane hydrochloride (WB4101, Tocris Cookson, Ballwin, Missouri), a selective antagonist of the α_{1A} adrenoceptor (Zhong and Minneman, 1999).

RESULTS

The body weight of the control and stressed rats was measured daily between 1400 and 1500. The control rats were 44.5 ± 1.5 g ($n = 24$) on PND 21 and 58.8 ± 1.9 g ($n = 24$) on PND 24 (Figure 1). The body weight of the stressed group was 44.2 ± 1.8 g ($n = 23$) before the first stress session on PND 21, and 51.0 ± 2.3 g ($n = 20$) after the last stress session, on PND 24. The difference in body weight between stressed and control rats was statistically significant after the second day of stress ($p < 0.01$). Stressed rats that were not used for electrophysiological experiments continued to display reduced body weight gain for as long as body weight was monitored (up to 10 days after stressor cessation, data not shown).

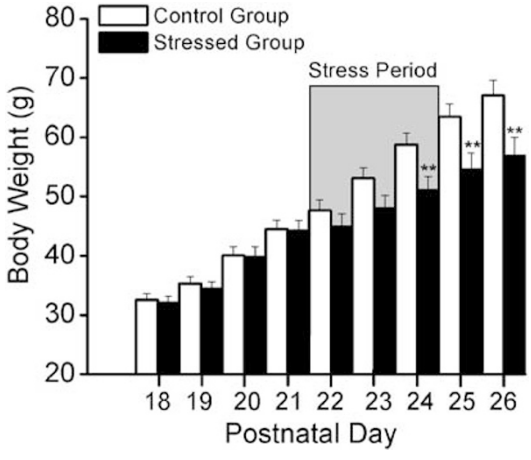


Figure 1 Restrain/tail-shock stress reduces body weight gain. Exposure to stress on PNDs 22, 23, and 24 reduced body weight gain. The body weight difference between control and stressed rats was statistically significant after the first day of stress (**$p < 0.01$). Data on PND 26 are from rats that were not used for electrophysiological experiments. Sample sizes range from 12 (PND 26) to 24 rats.

Stress Blocks Noradrenergic Facilitation of GABAergic Synaptic Transmission

Noradrenergic modulation of spontaneous IPSCs (sIPSCs). To investigate whether NE modulates GABAergic transmission in the BLA, and whether stress alters this modulation, we first examined the effects of NE on action-potential dependent, sIPSCs recorded from BLA pyramidal neurons, in control and stressed rats. sIPSCs were recorded at a holding potential of −70 mV, and in the presence of D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM), and yohimbine (20 μM) to block NMDA, AMPA/kainate, and β and α_2 receptors, respectively. In control rats, the mean frequency of sIPSCs recorded from the soma of BLA pyramidal neurons was 3.1 ± 1.6 Hz ($n = 21$). Bath application of bicuculline (10 μM) eliminated sIPSCs, confirming that they were mediated by $GABA_A$ receptors. NE at 1, 10, and 100 μM produced a dose-dependent enhancement in the frequency and amplitude of sIPSCs (Figure 2). At 100 μM of NE, the enhancement of sIPSCs was too high to be quantified precisely. The 10 μM concentration appeared to be close to the EC_{50}, and therefore it was used in subsequent experiments. After the application of 10 μM NE, the mean frequency of sIPSCs was increased to $984.39 \pm 148.2\%$ of the control values ($n = 21$, $p < 0.01$; Figure 3a). The amplitude of sIPSCs was increased to $144.0 \pm 12.8\%$ of the control values ($n = 21$, $p < 0.05$; Figure 3a). These effects persisted throughout the application of NE and were completely reversed after removal of the agonist. The effects of NE were not accompanied by any significant change in the rise time or decay time constant of sIPSCs (Figure 3a), and were blocked by the α_1 adrenoceptor antagonist prazosin (1 μM, Figure 3c), confirming that NE was acting via α_1 adrenergic receptors.

In stressed rats, the mean frequency of sIPSCs was 2.6 ± 2.3 Hz. NE (10 μM) had no significant effect on the frequency or amplitude of sIPSCs. Thus, in the presence of NE (10 μM), the frequency of sIPSCs was $128.9 \pm 19.2\%$ and the amplitude was $111.4 \pm 10.2\%$ of the control values ($n = 19$, Figure 3b). In addition, bath perfusion of NE (10 μM) caused no significant changes in the kinetics of these currents (rise time and decay time constant of sIPSCs; Figure 3b).

To identify the subtype of α_1 adrenoceptors involved in the effects of NE on control rats, we first applied NE (10 μM) in the additional presence of CEC (10 μM) and BMY 7378 (300 nM) to block α_{1B} and α_{1D} adrenoceptors. There was no significant attenuation of the effects of NE in the presence of these antagonists (Figure 4). Thus, NE increased the frequency of sIPSCs from 2.8 ± 2.4 to 27.1 ± 7.9 Hz ($p < 0.01$, $n = 6$; Figure 4), and the amplitude of sIPSCs to $154 \pm 11.3\%$ of the control values ($p < 0.05$, $n = 6$; Figure 4).

Next, we examined the effects of the specific α_{1A} adrenoceptor agonist A61603. In control rats, A61603 (1 μM) increased the frequency and amplitude of sIPSC to 1034 ± 158.6 and $162 \pm 14.2\%$ of the control values, respectively ($p < 0.01$, $n = 16$; Figure 5a). There were no effects on the rise time or the decay time constant of sIPSCs (Figure 5a). In stressed rats, A61603 had no significant effect (Figure 5b). Thus, in the presence of 1 μM A61603 the frequency of sIPSCs was $132 \pm 21\%$ and the amplitude of sIPSCs was $106 \pm 8.8\%$ of the control values ($n = 18$, Figure 5b). The effects of A61603 on sIPSCs in control rats were blocked by the selective α_{1A} adrenoceptor antagonist WB4101 (1 μM, Figures 5c and d).

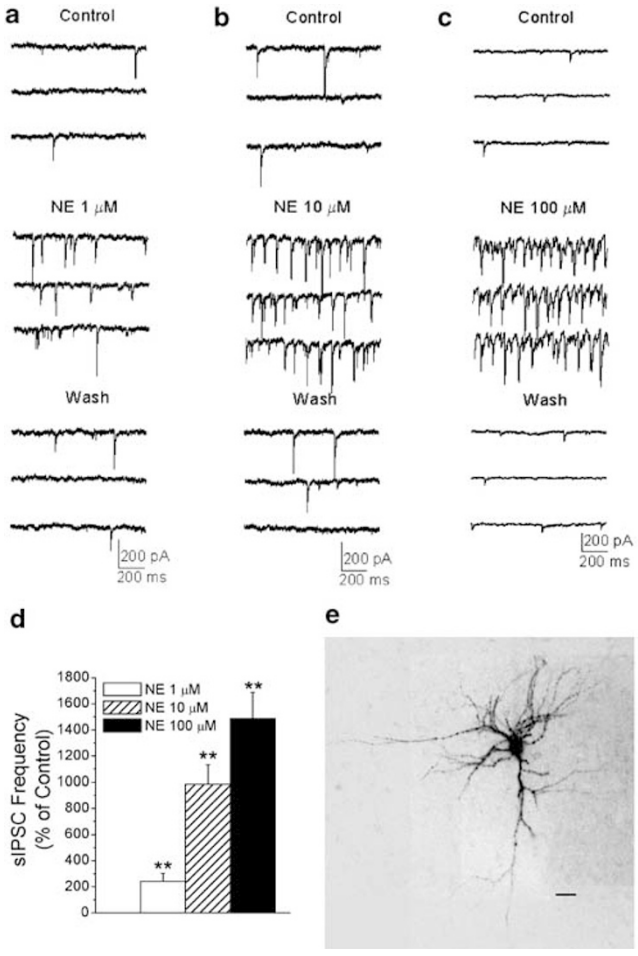
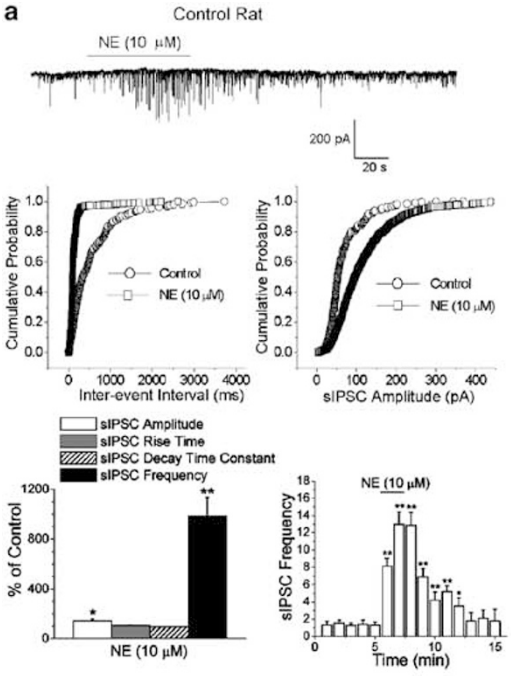
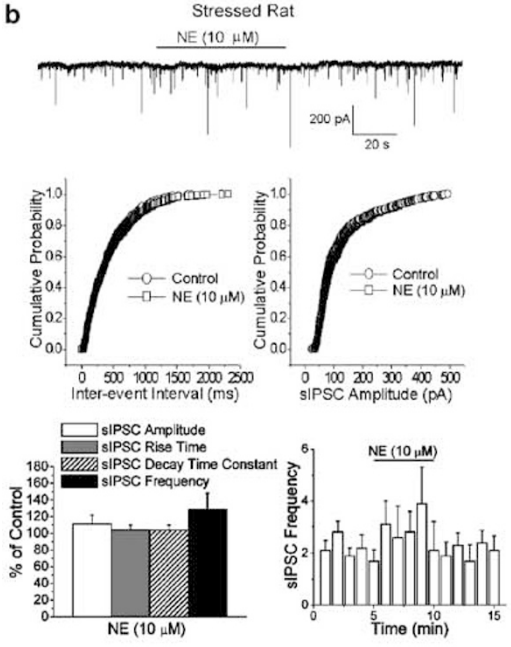


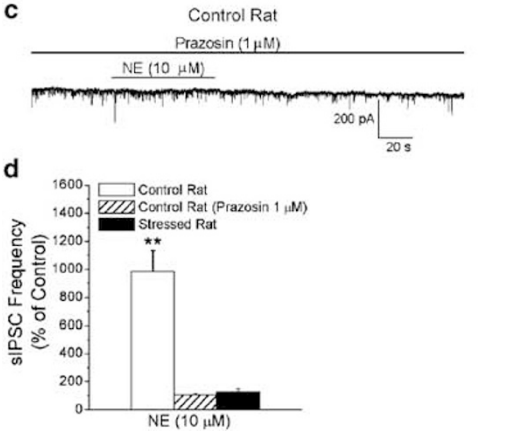
Figure 2 Activation of α_1 adrenoceptors increases tonic inhibition of BLA pyramidal neurons in a dose-dependent manner. (a–c) sIPSCs recorded from three different cells are shown. The holding potential is −70 mV. The medium contains D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM), and yohimbine (20 μM). The application of 1, 10, and 100 μM NE increased the frequency of sIPSCs in a dose-dependent manner. The bar graph (d) shows group data of the increase of sIPSC frequency ($n = 8$ for each concentration of NE, $^{**}p < 0.01$). (e) Photomicrograph of pyramidal cell (b) showing the typical morphology of the recorded neurons. The cell has been labeled with Lucifer Yellow. Scale bar, 40 μm.

Taken together, these results suggest that (1) NE, acting via α_{1A} adrenoceptors, enhances tonic inhibition of pyramidal cells in the BLA by inducing a massive increase in action potential-dependent spontaneous release of GABA, and (2) stress impairs this function of NE.

Noradrenergic modulation of evoked IPSCs (eIPSCs). It has been shown previously that NE reduces evoked inhibitory transmission in the hippocampus via α adrenoceptors (Madison and Nicoll, 1988; Doze *et al*, 1991). More recently, in the sensorimotor cortex, it was found that NE actually has a small facilitatory effect on eIPSCs, which is detected when $GABA_B$ receptors are blocked (Bennett *et al*, 1997). To determine the effects of NE on evoked inhibitory







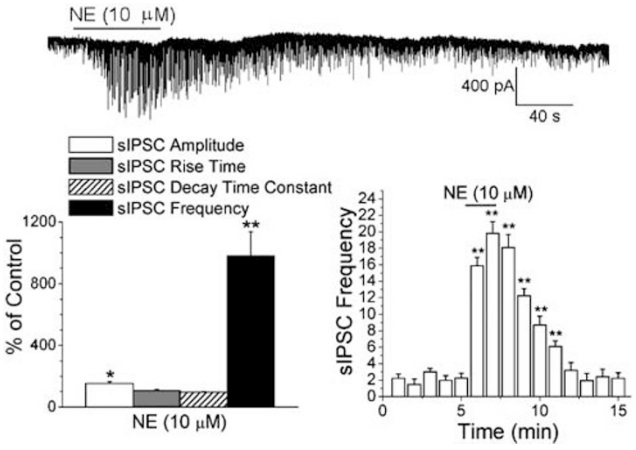
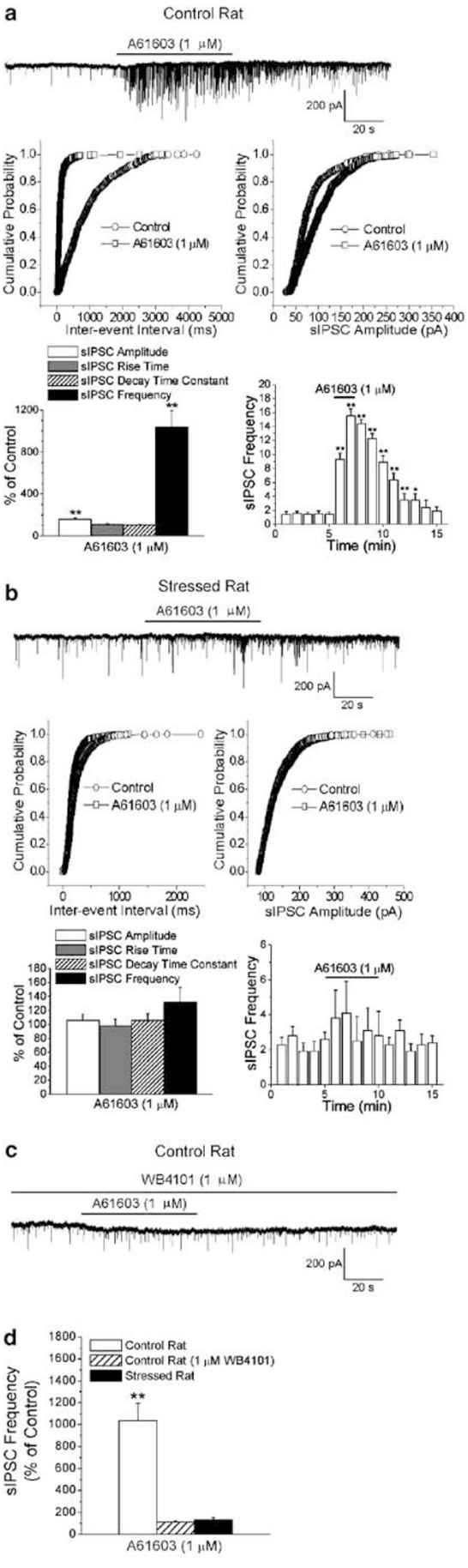


Figure 4 The NE-induced enhancement of sIPSCs is not blocked by α_{1B} and α_{1D} adrenoceptor antagonists. Top trace: sIPSCs recorded from a BLA pyramidal cell of a control rat (holding potential is −70 mV). Bath application of NE (10 μM) in the presence of D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM), yohimbine (20 μM), CEC (10 μM), and BMY 7378 (300 nM) reversibly increased the frequency and amplitude of sIPSCs. The bar graph shows pooled data (mean ± SEM) from six neurons. $^{*}p<0.05$, $^{**}p<0.01$.

transmission in the BLA we applied 10 μM NE while recording eIPSCs in control rats. In the absence of a $GABA_B$ receptor antagonist, NE (10 μM) reduced the amplitude of eIPSCs to 48.2 ± 10.3% of the control levels ($p<0.01$, $n=8$; Figure 6). However, in the presence of SCH50911 (20 μM), a specific antagonist of the $GABA_B$ receptors, NE enhanced the amplitude of eIPSCs to 162.4 ± 9.3% of the control, $p<0.01$, $n=10$; Figure 7a) without affecting the rise time and decay time constant of the eIPSCs (Figure 7a). Similar effects were obtained when α_{1A} adrenoceptors were activated by the application of 1 μM A61603 (Figure 7c). Thus, A61603 (1 μM) increased the amplitude of eIPSCs to 159.4 ± 10.7% of the control ($p<0.01$, $n=8$, Figure 7c) without affecting the kinetics of the eIPSCs (Figure 7c). The effects of the drugs were

Figure 3 Activation of α_1 adrenoceptors increases tonic inhibition of BLA pyramidal neurons in control rats, but not in stressed rats. (a) Top trace: effects of NE (10 μM) on sIPSCs recorded from a BLA pyramidal cell of a control rat. The holding potential is −70 mV. The medium contains D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM), and yohimbine (20 μM). Middle graphs: cumulative probability plots of interevent intervals and amplitude of sIPSCs, in control conditions and during NE perfusion (same cell as in the top trace). Bottom graphs: pooled data (mean ± SEM) from 21 neurons. The bar graph on the left shows the NE-induced changes in amplitude, frequency, and kinetics of sIPSCs. The bar graph on the right panel shows the time course of changes in sIPSC frequency during the application of NE. $^{*}p<0.05$, $^{**}p<0.01$. (b) Top trace: sIPSCs recorded from a BLA pyramidal cell of a stressed rat (the holding potential is −70 mV); NE (10 μM) had no significant effect. Middle graphs: cumulative probability plots of interevent intervals and amplitude of sIPSCs in control conditions and during NE perfusion (same cell as in the top trace). Bottom graphs: pooled data (mean ± SEM) from 19 neurons. Effects of NE on the amplitude, kinetics, and frequency of sIPSCs in stressed rats. (c) Prazosin (1 μM) prevented the NE-induced increase of sIPSCs observed in control rats. (d) The bar graph shows the effects of NE on the mean frequency of sIPSCs recorded from control rats (in the absence and in the presence of prazosin), and stressed rats (in the absence of prazosin). $^{*}p<0.05$, $^{**}p<0.01$.



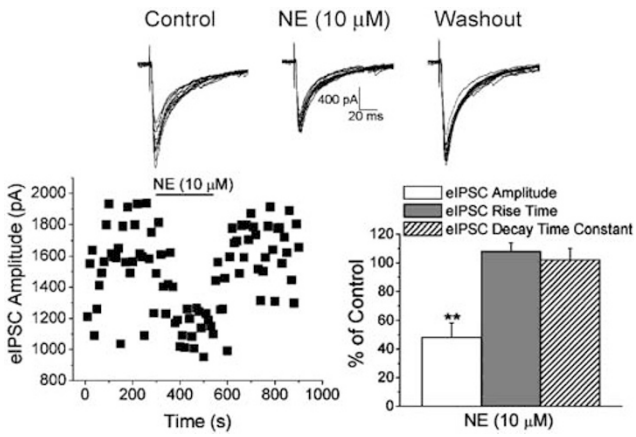


Figure 6 Activation of α_1 adrenoceptors reduces the amplitude of eIPSCs in control rats. Top traces: eIPSCs recorded from a BLA neuron of a control rat. The slice medium contains D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM), and yohimbine (20 μM). NE reduced the amplitude of eIPSCs with no significant effect on their kinetics. Bottom graphs: the plot shows the time course of the NE effects on the amplitude of eIPSCs (same cell as in top traces). The bar graph shows the relative (% of control) NE-induced changes in amplitude and kinetics of eIPSCs. Pooled data from eight neurons. $^{**}p<0.01$.

reversible. In stressed rats, neither NE nor A61603 had a significant effect on the amplitude, rise time, and decay time constant of eIPSCs (Figure 7b and d). In the presence of NE (10 μM), the eIPSC amplitude was 109 ± 8.2% of the control ($n=11$), and in the presence of A61603, the amplitude of the eIPSCs was 103 ± 7.4 % of the control ($n=10$). These results suggest that (1) NE facilitates evoked the GABAergic transmission via α_{1A} adrenergic receptors, (2) this facilitatory effect is masked due to the activation of presynaptic $GABA_B$ autoreceptors following the NE-induced enhancement of spontaneous GABA release, and (3) stress blocks the facilitatory effect of NE on evoked GABA release.

Noradrenergic modulation of mIPSCs. The enhancement of eIPSCs and action-potential-dependent sIPSCs by NE could be due to a depolarizing effect via the activation of

Figure 5 Activation of α_{1A} adrenoceptors increases tonic inhibition of BLA pyramidal neurons in control rats, but not in stressed rats. (a) Top trace: sIPSCs recorded from a BLA pyramidal cell of a control rat (the holding potential is −70 mV). Bath application of A61603 (1 μM), a specific α_{1A} adrenoceptor agonist, reversibly increased the frequency and amplitude of sIPSCs. The slice medium contains D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM), and yohimbine (20 μM). Middle graphs: cumulative probability plots of sIPSC interevent intervals and amplitude in control conditions and during A61603 perfusion (same cell as in the top trace). Bottom graphs: bar graphs show pooled data (mean ± SEM) from 16 neurons. (b) sIPSCs recorded from a BLA pyramidal cell of a stressed rat (the holding potential is −70 mV). Bath application of A61603 (1 μM) caused no significant change in the frequency or amplitude of sIPSCs. Middle graphs: cumulative probability plots of sIPSCs interevent intervals and amplitude in control conditions and during A61603 (1 μM) perfusion (same cell as in the top trace). Bottom graphs: bar graph shows pooled data (mean ± SEM) from 18 neurons. (c) WB4101 (1 μM) prevented the A61603-induced effects observed in control rats. (d) Bar graph shows the effects of A61603 (1 μM) on the mean frequency of sIPSCs recorded from control rats (in the absence and in the presence of WB4101), and stressed rats (in the absence of WB4101). $^{*}p<0.05$, $^{**}p<0.01$.

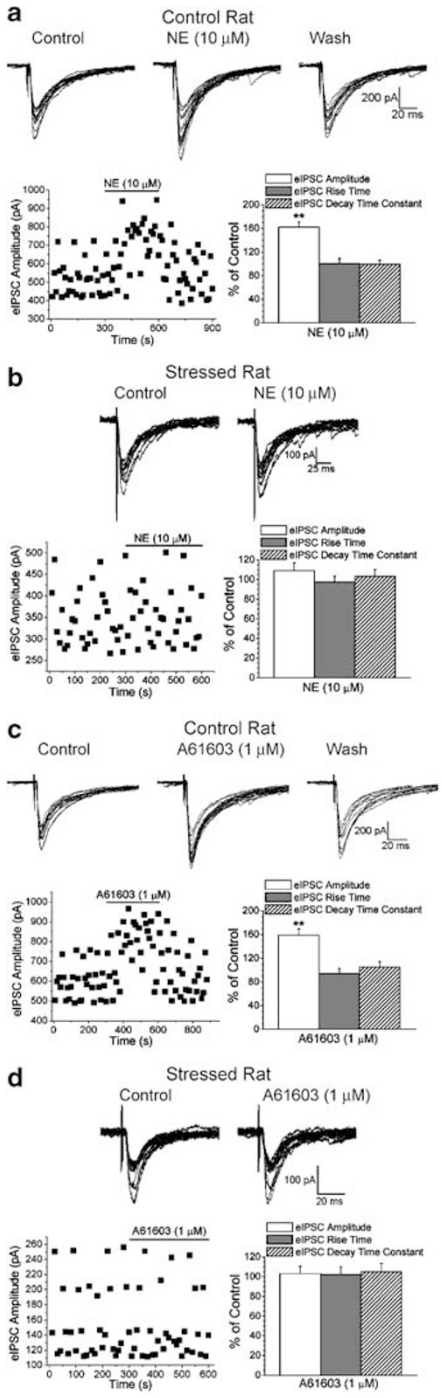


Figure 7 In the presence of a $GABA_B$ receptor antagonist, activation of α_{1A} adrenoceptors increases the amplitude of eIPSCs in control rats, but not in stressed rats. (a) Top traces: eIPSCs recorded from a BLA pyramidal cell of a control rat. In addition to D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM), and yohimbine (20 μM), the slice medium also contains 20 μM SCH50911. NE increased the amplitude of the eIPSCs, without affecting their kinetics. Bottom graphs: the plot shows the time course of the NE effect on eIPSC amplitude (same cell as in the top traces). The bar graph shows the effect of NE on the amplitude and kinetics of eIPSCs. Pooled data from 10 neurons. $^{**}p<0.01$. (b) Data similar to those shown in (a), but from stressed rats. The bar graph shows pooled data from 11 neurons. (c) In control rats, the α_{1A} agonist A61603 produced similar effects to those of NE. Top traces and bottom left plot show data from the same cell. The bar graph shows pooled data from eight BLA neurons. (d) In stressed rats, A61603 had no significant effects on eIPSCs. The bar graph shows pooled data from 10 BLA neurons.

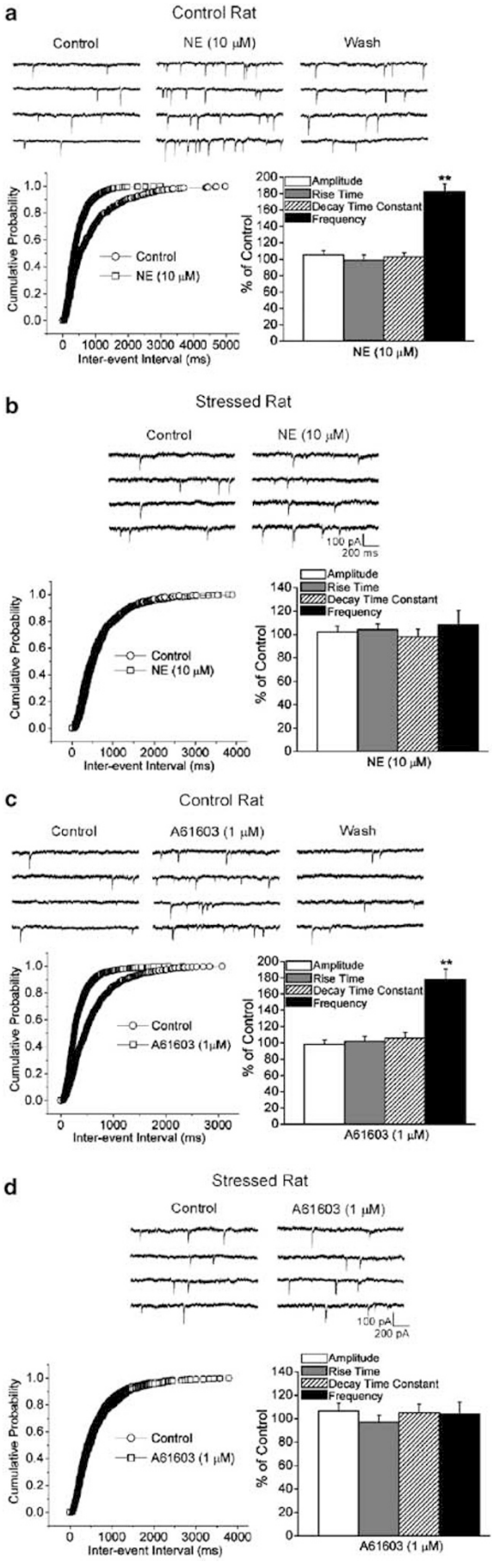
somatodendritic α_{1A} adrenoceptors on GABAergic neurons, and/or due to a direct effect at GABAergic terminals. To determine whether NE modulates GABA release by a direct effect on GABAergic terminals in the BLA, we tested the effects of NE on mIPSCs, which do not depend on the presynaptic invasion of action potentials or Ca^{2+} influx. mIPSCs were recorded in a medium containing D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM), yohimbine (20 μM), and TTX (1 μM). In the absence of NE, the frequency of mIPSCs was 0.68 ± 0.32 Hz and their amplitude was 114.0 ± 12 pA ($n=10$). NE (10 μM) increased the frequency of mIPSCs to 182.3 ± 9.6 % of the control levels ($p<0.01$, $n=10$; Figure 8a). The amplitude, rise time, and decay time constant of the mIPSCs were not significantly affected by 10 μM NE (Figure 8a). Similar effects were observed after the application of the α_{1A}-specific agonist A61603 (Figure 8c). A61603 (1 μM) increased the frequency of mIPSCs from 0.71 ± 0.24 to 1.28 ± 0.31 (178 ± 12.4% of the control, $p<0.01$, $n=9$; Figure 8c). The amplitude and kinetics of mIPSCs were not affected by A61603 (Figure 8c).

In stressed rats, neither NE (10 μM) nor A61603 (1 μM) produced a significant effect on mIPSCs frequency, amplitude, or kinetics (Figure 8b and d). Thus, the frequency of mIPSCs was 0.68 ± 0.25 and 0.64 ± 0.34 Hz before and during the application of NE, respectively ($n=10$), and 0.72 ± 0.27 and 0.63 ± 0.31 Hz in the presence and absence of 1 μM A61603, respectively ($n=8$).

These results suggest that (1) NE facilitates GABA release by a direct effect on GABAergic terminals, and (2) this mechanism of noradrenergic facilitation of GABA release is impaired by stress.

Facilitation of GABAergic Transmission by α_{1A} Adrenoceptors is Mediated by Phospholipase C (PLC)

Studies in other brain regions or cell types have shown that α_1 adrenoceptors are coupled to PLC via a G-protein, and can increase the intracellular calcium concentration $[Ca^{2+}]_i$ by mobilizing Ca^{2+} from intracellular stores, as well as by increasing the Ca^{2+} influx (Schwinn *et al*, 1991; Wu *et al*, 1992; Cohen and Almazan, 1993; Lepretre *et al*, 1994; Kulik *et al*, 1999). However, certain effects of α_{1A} activation involve signaling pathways that are independent of PLC activation and intracellular Ca^{2+} rise (Berts *et al*, 1999). To determine whether the α_{1A} adrenoceptor-mediated facilitation of GABA release, in the BLA, involves the activation of PLC, we examined whether the effects of NE on the GABAergic transmission are blocked by a PLC inhibitor. In control rats, NE (10 μM) or A61603 (1 μM) enhanced the frequency and amplitude of sIPSCs in the presence of U73343 (20 μM), the inactive isomer of the PLC inhibitor U73122, but had no effects in the presence of 20 μM U73122 (Figure 9). Thus, in the presence of U73343, NE increased the frequency of sIPSCs to 1022.8 ± 105.3% of the control levels ($p<0.01$, $n=8$; Figure 9a) and increased the amplitude of sIPSCs to 161 ± 11.7% ($p<0.01$, $n=6$; Figure 9a); A61603 (1 μM) increased the frequency of sIPSCs to 978.1 ± 102.1% ($p<0.01$, $n=8$; Figure 9b), and increased the amplitude of sIPSCs to 154 ± 12.3% of the control levels ($p<0.01$, $n=8$; Figure 9b). In contrast, in the presence of U73122 (20 μM), NE (10 μM) and A61603 (1 μM) failed to induce any significant changes in the frequency



and amplitude of sIPSCs (Figure 9c–e). Similarly, the effects of NE (10 μM) on the amplitude of eIPSCs, as well as on the frequency of mIPSCs, were blocked by 20 μM U73122 (not shown).

Stress Blocks α_{1A} Adrenoceptor-Mediated Suppression of BLA Field Potentials

Since the activation of α_{1A} adrenoceptors facilitates GABAergic transmission, the function of these receptors at the network level could be to dampen neuronal excitability and responsiveness. However, while spontaneous GABAergic activity is dramatically enhanced by activation of α_{1A} adrenoceptors (Figure 5), evoked GABAergic transmission is suppressed due to presynaptic inhibition of GABA release via $GABA_B$ autoreceptors (Figure 6). Therefore, under physiological conditions when $GABA_B$ receptors are not blocked, α_{1A} adrenoceptor activation could enhance the amygdala's responsiveness (due to the reduction in evoked GABA release), unless the enhancement of spontaneously released extracellular GABA plays a more decisive role in neuronal excitability. To determine the net effect of α_{1A} adrenoceptor activation on neuronal responsiveness and excitability in the BLA, and whether this effect is altered by stress, we investigated the effects of NE or A61603 on population, field responses, in the absence of $GABA_B$ receptor blockade, in control and stressed rats.

Field potentials in the BLA were evoked by stimulation of the external capsule. These responses consist of one major, negative component that corresponds in time course to the EPSP recorded intracellularly from BLA pyramidal cells (Aroniadou-Anderjaska *et al*, 2001; Chen *et al*, 2003), and is mediated by AMPA/kainate receptors (Aroniadou-Anderjaska *et al*, 2001). In control rats, 10 μM NE, in the presence of propranolol (10 μM) and yohimbine (20 μM), produced a significant reduction in the peak amplitude of evoked field potentials (83.8 ± 5.3% of control levels, $n = 14$, $p < 0.05$; Figure 10a). Similarly, bath application of 1 μM A61603 caused a significant reduction in the peak amplitude of the field potentials to 83.1 ± 5.2% of the control levels ($p < 0.05$, $n = 12$; Figure 10b). In contrast, in stressed rats, neither NE (10 μM) nor A61603 (1 μM) had a significant effect on the amplitude of the field potentials (Figure 10, bottom panels).

These results suggest that the function of α_{1A} adrenoceptors in the BLA is to reduce neuronal excitability/responsiveness, and this function is impaired by stress.

Figure 8 Activation of α_{1A} adrenoceptors increases the frequency of mIPSCs in control rats, but not in stressed rats. mIPSCs were recorded in the presence of TTX (1 μM), D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM), and yohimbine (20 μM). (a) Top traces: mIPSCs recorded from a BLA pyramidal neuron of a control rat. NE (10 μM) increased the frequency of mIPSCs. Bottom graph: the left panel shows the cumulative probability plots of interevent intervals of mIPSCs under control conditions and during the application of NE (same cell as in top traces). The bar graph shows the effect of NE on the amplitude, kinetics, and frequency of mIPSCs. Pooled data from 10 neurons, $^{**}p < 0.01$. (b) Similar data to those shown in (a), but from stressed rats. NE had no significant effect on mIPSCs. The bar graph shows pooled data from 10 neurons. (c) In control rats, the α_{1A} antagonist A61603 had similar effects to those induced by NE. The bar graph shows pooled data from nine BLA neurons. (d) A61603 had no significant effects on mIPSCs recorded from BLA pyramidal cells of stressed rats. The bar graph shows pooled data from eight cells.

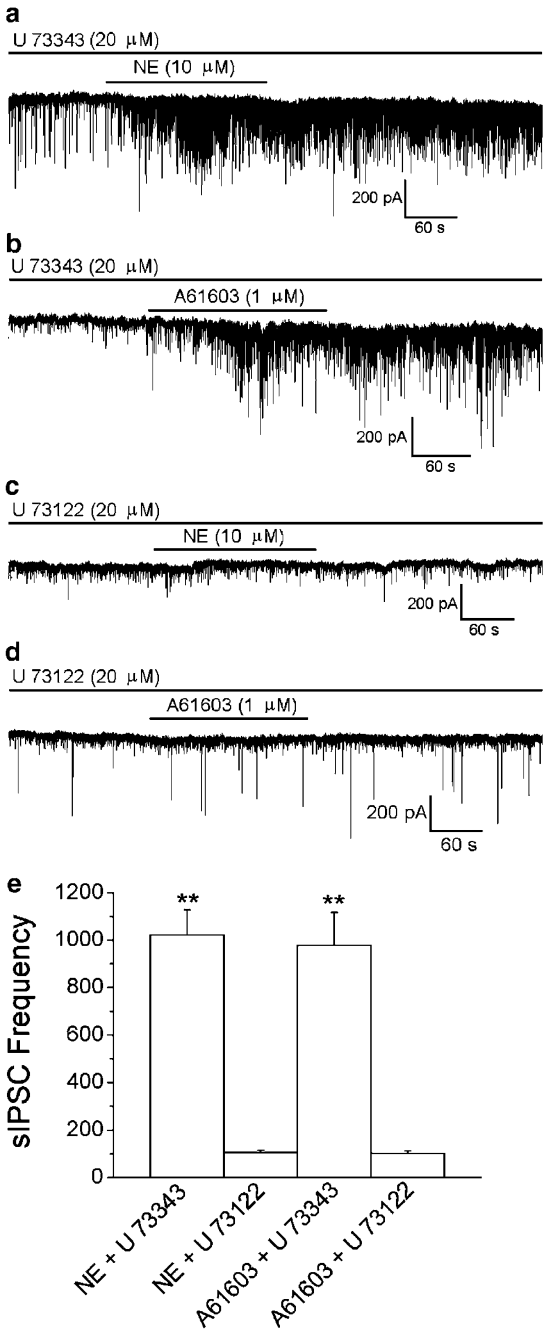


Figure 9 α_{1A} adrenoceptors in the BLA are coupled to PLC. (a–d) sIPSCs recorded from BLA pyramidal neurons. NE (a) or A61603 (b) increased the frequency and amplitude of sIPSCs in the presence of the inactive isomer of a PLC inhibitor (U73343), but had no effect in the presence of the PLC inhibitor U73122 (c and d). The slice medium contains D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM), and yohimbine (20 μM). (e) Bar graphs showing the effects of NE (10 μM) or A61603 (1 μM) on the frequency of sIPSCs in the presence of U73343 or U73122. Pooled data from eight neurons.

DISCUSSION

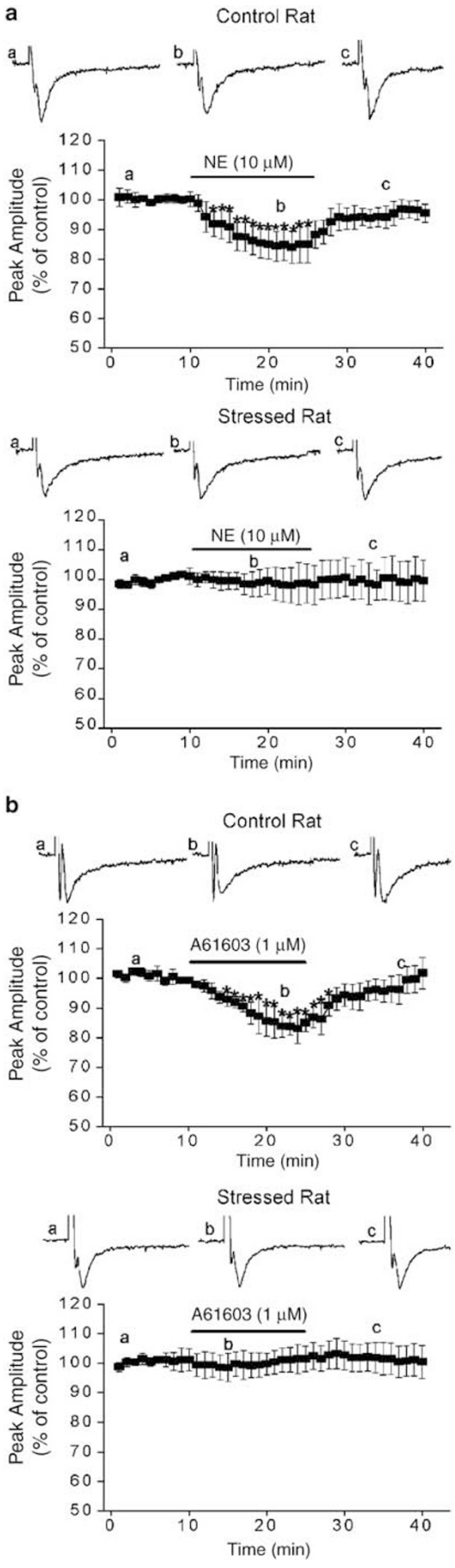
The present study describes two main findings. First, activation of the α_{1A} subtype of adrenergic receptors facilitates both tonic and phasic $GABA_A$ receptor-mediated inhibition of BLA pyramidal neurons. Second, stress produces a severe impairment of the α_{1A} adrenoceptor-mediated facilitation of GABAergic synaptic transmission in the BLA. These findings provide one possible explanation for (1) the antiepileptic effects of NE in temporal lobe epilepsy, (2) the amygdala's hyperexcitability in stress-related affective disorders, and (3) the stress-induced increase in the frequency of seizures in epileptic patients.

NE Facilitates GABAergic Transmission in the BLA via presynaptic α_{1A} Adrenoceptors

All three subtypes of α_1 adrenoceptors, α_{1A}, α_{1B}, and α_{1D}, are present in the amygdala, as determined by *in situ* hybridization (Day *et al*, 1997). The distribution of these receptors varies in different nuclei of the amygdala. The BLA expresses the α_{1A} adrenoceptor subtype almost exclusively (Day *et al*, 1997; Domyancic and Morilak, 1997). The role of these receptors in the amygdala's physiology and function has been unknown. In the present study, we show that NE, acting via the α_{1A} subtype of adrenergic receptors, facilitates GABA release in the BLA. Spontaneous, evoked, and quantal release of GABA were enhanced by NE or the specific α_{1A} adrenoceptor agonist A61603.

Endogenous NE released from noradrenergic terminals reaches its targets both by diffusion and via conventional synapses (Papadopoulos and Parnavelas, 1990; Seguela *et al*, 1990; Asan, 1993; Arce *et al*, 1994; Li *et al*, 2002). In the BLA, noradrenergic axons form asymmetric synapses with the dendrites of GABAergic neurons (Li *et al*, 2002). Although α_{1A} adrenoceptors may be located in such dendritic synapses and could be involved in the enhancement of spontaneous and evoked GABA release by NE, the increase in the frequency of mIPSCs by α_{1A} adrenoceptor activation indicates the presence of these receptors on GABAergic terminals. The enhancement of spontaneous GABA release by NE has also been observed in other brain regions (Madison and Nicoll, 1988; Doze *et al*, 1991; Gellman and Aghajanian, 1993; Alreja and Liu, 1996; Bergles *et al*, 1996; Bennett *et al*, 1997, 1998; Kawaguchi and Shindou, 1998), and it is mediated via α_1 adrenoceptors (Gellman and Aghajanian, 1993; Alreja and Liu, 1996; Bergles *et al*, 1996; Kawaguchi and Shindou, 1998); the specific α_1 receptor subtype involved has not been determined. At least in the CA1 hippocampal area, it appears that α_1 adrenoceptors are located only on somatodendritic regions of GABAergic cells, since mIPSCs are unaffected by adrenergic agonists (Bergles *et al*, 1996). Thus, the amygdala and the hippocampus may differ in the subcellular distribution of α_1 adrenoceptors mediating the facilitation of GABA release.

Evoked GABA release in the hippocampus is suppressed by NE, and this effect is also mediated via α adrenoceptors (Madison and Nicoll, 1988). However, a similar effect of NE in the sensorimotor cortex has been found to be due to the activation of presynaptic $GABA_B$ autoreceptors; when $GABA_B$ receptors were blocked, NE enhanced evoked GABAergic transmission (Bennett *et al*, 1997). Similarly, in the present study, the facilitatory effect of NE on evoked GABAergic transmission was revealed only when $GABA_B$ receptors were blocked, suggesting that the accumulation of extracellular GABA due to the NE-induced enhancement of spontaneous GABA release inhibited evoked GABA release.



Since NE enhances spontaneous GABA release, but suppresses evoked GABA release when $GABA_B$ receptors are functional, this raises the question of what would be the net effect of α_{1A} adrenoceptor activation on the overall excitability and responsiveness of the amygdala. The BLA field potentials were reduced by NE or A61603 in the absence of $GABA_B$ receptor antagonists. It is unlikely that this effect is due to a reduction in glutamate release, because glutamatergic transmission in the BLA is suppressed via α_2, but not α_1 adrenoceptor activation (Ferry *et al*, 1997). Thus, the reduction of the BLA field potentials by NE or A61603 suggests that the dramatic enhancement of spontaneously released GABA induced by α_{1A} adrenoceptor activation (Figure 5) over-rides the reduction in evoked GABAergic transmission (Figure 7) producing a suppression of the amygdala's excitability.

The intracellular signaling mechanisms that mediate the physiological effects of α_{1A} adrenoceptor activation in the BLA involve the activation of PLC, since a PLC inhibitor prevented the enhancement of sIPSCs, eIPSCs, and mIPSCs by NE and A61603. The activation of PLC may lead to mobilization of Ca^{2+} from intracellular stores, and/or Ca^{2+} influx, following phosphoinositide hydrolysis and formation of IP3, as it has been observed in different tissues and cell types following α_1 adrenoceptor activation (Schoepp and Rutledge, 1985; Schwinn *et al*, 1991; Perez *et al*, 1993; Kulik *et al*, 1999; Zhong and Minneman, 1999; Khorchid *et al*, 2002), or α_{1A} adrenoreceptor activation (Cohen and Almazan, 1993; Lepretre *et al*, 1994). In the present study, since NE or A61603 enhanced the frequency of mIPSCs, the influx of Ca^{2+} through voltage-gated calcium channels is not necessary for the α_{1A} adrenoreceptor-mediated facilitation of GABA release in the BLA.

The amygdala is a key player in the pathogenesis and symptomatology of temporal lobe epilepsy (Gloor, 1992; Weiss *et al*, 2000; Avoli *et al*, 2002). NE has long been known to display anticonvulsant properties, but little is known about the underlying mechanisms (Chen *et al*, 1954; Stanton, 1992; Stanton *et al*, 1992; Szot *et al*, 1999; Stoop *et al*, 2000; Weinshenker *et al*, 2001) The α_{1A} adrenoreceptor-mediated facilitation of GABA release in the BLA may be one of the mechanisms involved in the antiepileptic effects of NE in temporal lobe seizure disorders.

Stress Impairs the Function of α_{1A} Adrenoceptors in the BLA

Previous studies have suggested that excessive or repeated stress can produce long-lasting alterations in the amygdala's structure and function. Thus, chronic immobilization, in rats, induces hypertrophy of the dendritic arborizations of

Figure 10 Activation of α_{1A} adrenoceptors reduces BLA field potentials in control rats, but not in stressed rats. (a) Changes in the peak amplitude of BLA field potentials evoked by stimulation of the external capsule, in response to bath application of 10 M NE, in control (top panel, $n=9$) and stressed (bottom panel, $n=10$) rats. The medium contains propranolol (10 M) and yohimbine (20 M). (b) Similar data to those in (a), except that A61603 is applied in place of the NE. Pooled data from 10 slices (control rats, top panel) and eight slices (stressed rats, bottom panel). The slice medium same as in (a). Asterisks over error bars denote statistically significant reduction ($p<0.05$).

pyramidal and stellate neurons in the BLA (Vyas *et al*, 2002; Pawlak *et al*, 2003). Fear conditioning or other types of stressors such as exposure to a predator produce long-lasting changes in the efficacy of synaptic transmission in the amygdala (LeDoux, 1992; Davis *et al*, 1994; Rogan *et al*, 1997, McKernan and Shinnick-Gallagher, 1997; Adamec *et al*, 2001). In human patients with stress-related affective disorders, the amygdala exhibits hypertrophy (Strakowski *et al*, 1999; Altshuler *et al*, 2000), increased levels of basal activity (Drevets, 1999), or exaggerated responses to fearful stimuli (Rauch *et al*, 2000). In the present study, repeated restrain/tail-shock stress produced a severe impairment in the α_{1A} adrenoreceptor-mediated facilitation of GABA release in the BLA, indicating that stress impairs the function of α_{1A} adrenoceptors. This impairment could result from receptor desensitization, internalization, or down-regulation, or by an effect on the intracellular signaling pathways activated by PLC. In other brain regions, repeated stress reduces mRNA levels of α_1 adrenoceptors (Miyahara *et al*, 1999). Adrenergic receptors desensitize or undergo downregulation following prolonged exposure to the agonist (Yang *et al*, 1999; Chalothorn *et al*, 2002). Thus, during stress exposure, excessive release of NE in the amygdala (Galvez *et al*, 1996; Quirarte *et al*, 1998; Tanaka *et al*, 2000) may be responsible for the impairment of the α_{1A} adrenoreceptor function. In addition, previous studies have shown that restrain/tail-shock stress elevates plasma corticosterone levels (Servatius *et al*, 1995). Glucocorticoid receptors colocalize with α_1 adrenoceptors (Fuxe *et al*, 1985; Williams *et al*, 1997), and it has been demonstrated that corticosterone downregulates α adrenoceptors (Stone *et al*, 1986, 1987; Joels and de Kloet, 1989). Therefore, another possibility is that the corticosterone released during exposure to stress downregulates α_{1A} adrenoceptors. An important question is whether the impairment in the α_{1A} adrenoceptor function is a transient or a long-term effect. The investigations described here focus on changes measured within a relatively short period of time after stressor cessation. However, preliminary experiments have revealed differences in the α_{1A} adrenoceptor function between stressed and control rats on the fifth day after the termination of stress exposure, suggesting that the stress-induced dysfunction in the noradrenergic modulation of GABA release is not likely to be a short-term effect.

Functional implications. What are the possible functional implications of a stress-induced loss of the α_{1A} adrenoceptor-mediated noradrenergic facilitation of GABA release in the BLA? In the normal amygdala, basal levels of NE, acting via α_{1A} adrenoceptors, may contribute to tonic inhibition of BLA pyramidal neurons, by facilitating both action potential-dependent and -independent GABA release. The loss or impairment of this facilitation would result in hyperexcitability at rest, and a lower threshold of activation. When the normal amygdala is activated in response to an emotionally significant event triggering the release of NE, activation of α_{1A} adrenoceptors will facilitate the role of inhibitory transmission in active neuronal circuits; this role is not only to prevent overexcitation, but also to shape and sharpen the flow of excitatory activity. Therefore, loss of the α_{1A} adrenoceptor-mediated facilitation of synaptic inhibition may result in inappropriate overactivation of the amygdala and impairment in the processing and interpretation of an emotional stimulus. A dysfunction of this nature may also affect the formation of emotional memories. In the normal amygdala, noradrenergic facilitation of GABAergic transmission may either suppress memory formation (due to the suppression of excitation), or facilitate optimal registration of the memory trace (by regulating the level and flow of excitatory activity). In a hyper-responsive amygdala, when noradrenergic facilitation of GABA release is impaired, events of little emotional significance may be registered as significant, and memories of emotionally significant events may be 'overconsolidated'. It should be noted, however, that the net effect of stress on the function of the noradrenergic system in the BLA remains to be determined, as stress may also induce changes in the interaction of NE with other adrenoceptor subtypes (β and α_2) or neurotransmitter systems.

It has been hypothesized that the hyperactivity and hyper-responsiveness of the amygdala associated with certain affective disorders, such as PTSD, is due to the loss of proper cortical modulation of the amygdala, and/or due to an intrinsic lower threshold of amygdala response to emotionally significant stimuli (Villarreal and King, 2001). The present findings suggest that a reduction in GABAergic transmission due to the loss of the α_{1A} adrenoceptor-mediated facilitation of GABA release may be one of the mechanisms responsible for the apparently reduced threshold of amygdala's activation in these affective disorders. The present findings also suggest that a stress-induced impairment in the function of α_{1A} adrenoceptors, which could result in reduced tonic inhibition in the BLA, may be one of the mechanisms underlying the stress-induced increased frequency of seizures in patients with temporal lobe epilepsy (Temkin and Davis, 1984; Frucht *et al*, 2000). Moreover, our results suggest that the reduced central α_1 adrenoceptor responsiveness (Asnis *et al*, 1985, 1992), and binding (Crow *et al*, 1984; Gross-Isseroff *et al*, 1990) in depressed patients may be stress-related, and that one of the physiological consequences of this reduction is an impaired modulation of the GABAergic transmission.

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