Pre- and Postsynaptic Effects of Norepinephrine on γ-Aminobutyric Acid-Mediated Synaptic Transmission in Layer 2/3 of the Rat Auditory Cortex

HUMBERTO SALGADO,[1,2] FRANCISCO GARCIA-OSCOS,[1] LAURA MARTINOLICH,[1] SHAWN HALL,[3] ROBERT RESTOM,[3] KUEI Y. TSENG,[4] AND MARCO ATZORI[1]*

[1]*University of Texas at Dallas, School of Behavioral and Brain Sciences, Richardson, Texas*
[2]*Universidad Autonoma de Yucatan, Centro de Investigaciones Regionales Dr. Hideyo Noguchi, Merida, Yucatan, Mexico 97000*
[3]*University of Texas at Dallas, School of Natural Sciences, Richardson, Texas*
[4]*Department of Cellular and Molecular Pharmacology, Rosalind Franklin University of Medicine and Science/ The Chicago Medical School, North Chicago, IL*

KEY WORDS inhibition; pair recording; adrenoceptors; muscimol; patch-clamp

ABSTRACT Noradrenergic terminals from the locus coeruleus release norepinephrine (NE) throughout most brain areas, including the auditory cortex, where they affect neural processing by modulating numerous cellular properties including the inhibitory γ-aminobutyric acid (GABA)ergic transmission. We recently demonstrated that NE affects GABAergic signaling onto cortical pyramidal cells in a complex manner. In this study, we used a combination of patch-clamp recording and immunohistochemical techniques to identify the synaptic site and the location of the adrenergic receptors involved in the modulation of GABAergic signaling in cortical layer 2/3 of the rat. Our results showed that NE increases the frequency of spike-independent miniature inhibitory postsynaptic currents (mIPSCs), as well as the probability of release of unitary inhibitory postsynaptic currents (IPSCs) obtained with patch-clamp pair-recordings. The pharmacology of mIPSCs and the identification of adrenergic receptors in neurons containing the GABAergic marker parvalbumin (PV) suggest that NE increases the presynaptic probability of GABA release by activating α_2- and β-receptors on PV-positive neurons. On the contrary, bath-applied NE or phenylephrine, decreased the current mediated by pressure application of the $GABA_A$-receptor agonist muscimol, as well as the amplitude—but not the frequency—of mIPSCs, indicating that activation of postsynaptic α_1 adrenoceptors reversibly depressed GABAergic currents. We speculate that while a generalized postsynaptic decrease of GABAergic inhibition might decrease the synaptic activation threshold for pyramidal neurons corresponding to an alert state, NE might promote perception and sensory binding by facilitating lateral inhibition as well as the production of γ-oscillations by a selective enhancement of perisomatic inhibition. **Synapse 00:000–000, 2011.**

INTRODUCTION

The noradrenergic system that originates from the brainstem projects to widespread brain areas including the totality of the cortical mantle. From here, norepinephrine (NE) controls complex cognitive functions including perception, alertness, and attention. Both clinical and behavioral data suggest a role of the noradrenergic system in the pathophysiology of attention (Sunohara et al., 1999). In fact, selective noradrenergic drugs like atomoxetine or other NE-re-uptake inhibitors, as well as less selective pro-noradrenergic drugs like amphetamines are somehow effective in the treatment of attention deficit disorder (Gilbert et al., 2006; Sunohara et al., 1999). Dysregulation of

Contract grant sponsor: NIH/NIDCD; Contract grant number: R01DC005986; Contract grant sponsor: CONACyT; Contract grant number: MOD-ORD-1-09 PCI-047-11-09; Contract grant sponsors: NARSAD Young Investigator Award and School for Behavioral and Brain Sciences

*Correspondence to: Marco Atzori, Laboratory of Cell and Synaptic Physiology, School of Behavioral and Brain Sciences/GR41, University of Texas at Dallas, Richardson, TX 75080, USA. E-mail: marco.atzori@utdallas.edu

Received 15 March 2011; Revision 26 July 2011; Accepted 25 August 2011

DOI 10.1002/syn.20979

Published online 9 September 2011 in Wiley Online Library (wileyonlinelibrary.com).

cortical inhibitory interneurons releasing γ-aminobutyric acid (GABA) has been hypothesized to underlie a series of neurological and psychiatric disorders like schizophrenia and autism (Hoftman and Lewis, 2011; Uhlhaas and Singer, 2010). In particular, a down-regulation of parvalbumin-positive GABAergic interneurons in cortical circuits, together with a decrease in the level of different isoforms of the GABA-synthesizing enzyme glutamic acid decarboxylase are among the few consistent findings from postmortem brains of psychotic patients (Beneyto et al., 2010; Hoftman and Lewis, 2011). Together, these findings suggest that the noradrenergic modulation of GABAergic transmission might be a critical regulatory component of the cortical contribution to attention, perception and other cognitive processes.

Several in vivo and in vitro studies have shown that the auditory cortex is subject to an important noradrenergic modulation, whose cellular mechanisms have not been completely understood (Foote et al., 1975; Manunta and Edeline, 1997, 1998, 1999, 2000, 2004; Salgado et al., 2011a,b). We recently demonstrated that NE can exert both facilitatory and inhibitory effects on local GABAergic synaptic transmission onto layer 2/3 pyramidal neurons of the auditory cortex (Salgado et al., 2011a, b), whereby activation of α_2 or β adrenoceptors enhanced, while stimulation of α_1-adrenoceptors suppressed inhibitory postsynaptic currents (IPSCs). This study used electrophysiological and immunochemical methods aimed to identify the synaptic site of action of the adrenoceptors involved in the modulation of cortical GABAergic signaling.

MATERIALS AND METHODS

Animals

All experimental procedures were conducted according to the National Institutes of Health Guidelines (UTD IACUC 04-04). Sprague Dawley rats (Charles River, Wilmington, MA) were housed in a facility with controlled humidity and temperature (24°C), fed ad libitum, and maintained under a 12–12 h light-dark cycle.

Slice preparation

We used an auditory cortex slice preparation similar to one previously described (Atzori et al., 2001). Twenty-three to 35 days-old rats were anesthetized with isoflurane (Baxter, Round Lake IL), sacrificed and their brains sliced with a vibrotome (VT1000, Leica, Germany) in a cold solution (0–4°C) containing (in mM): 126 NaCl, 3.5 KCl, 10 Glucose, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 1.5 $CaCl_2$, 1.5 $MgCl_2$, and 0.2 ascorbic acid, at pH 7.4 and saturated with a mixture of 95% O_2 and 5% CO_2 (artificail cerebro-spinal fluid, ACSF). Two hundred and seventy micrometer thick coronal slices from the most caudal fourth of the brain were retained after removing the occipital convexity, and subsequently incubated in ACSF at 32°C before being placed in the recording chamber. The recording area was selected dorsally to the rhinal fissure corresponding to the auditory cortex (Rutkowski et al., 2003). The recording solution had an osmolarity of ≈ 305 mOsm, and also contained 6,7-Dinitroquinoxaline-2,3-dione (DNQX, 10 μM) and kynurenate (2 mM) or amino-5-phosphonovaleric acid (APV, 100 μM) for blocking α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR)- and *N*-methyl-D-aspartate receptor (NMDAR)-mediated transmission, respectively.

Electrophysiology

Slices were placed in an immersion chamber, where cells from cortical layer 2/3 were visually selected using a BX51 (Olympus, Japan) with an infrared camera system (DAGE-MTI, Michigan City, IN). Inhibitory postsynaptic currents (IPSCs) were recorded in the whole-cell configuration in voltage-clamp mode using a pipette solution containing (mM): 100 CsCl, 5 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid K (BAPTA-K), 1 lidocaine *N*-ethyl bromide (QX314), 1 $MgCl_2$, 10 N-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES), 4 glutathione, 3 ATPMg, 0.3 $GTPNa_2$, 20 phosphocreatine, titrated to pH 7.2 and with osmolarity ≈ 270 mOsm. The holding potential was −60 mV not corrected for the junction potential (≈ 4 mV). A 2 mV voltage step was applied at the beginning of every episode to monitor the quality of the recording. Access resistance (10–20 MΩ) was constantly monitored and remained stable during all the experiments (<20%). All signals were filtered at 2 KHz and sampled at 10 KHz. All experiments were performed a room temperature (RT) (20–23°C).

Miniature IPSCs (mIPSC) were recorded in the presence of the Na-channel blocker tetrodotoxin (TTX, 1 μM) using the same intracellular solution used in the postsynaptic cell for pair recordings. Each cell analyzed had at least 500 miniature synaptic events per condition. As above, all experiments were performed in the presence of the AMPA- and NMDA-receptor blockers DNQX (10 μM) and kynurenate (2 mM) to block glutamatergic synaptic currents.

Abbreviations

APV	amino phosphovaleric acid
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
cIPSC	chemically evoked IP
DNQX	6,7-dinitroquinoxaline-2,3-dione
eIPSC	electrically evoked IPSC
GABA	γ-aminobutyric acid
IN	interneuron
IPSC	inhibitory postsynaptic current
NE	norepinephrine
NMDA	*N*-methyl-D-aspartate
PV	parvalbumin
uIPSC	unitary IPSC.

$GABA_A$ receptors ($GABA_AR$) were activated by brief applications of the $GABA_AR$ agonist muscimol (100 μM) at 100–200 μm from the recording areas once every 30–40 s. A stock solution of muscimol was diluted 10-fold in ACSF before being back-filled to a glass pipette similar to the one used for recording. Muscimol application was performed with a pressure system (picospritzer, General Valve Corp. Fairfield, NJ) through a glass pipette (≅ 25 psi, 3–12 ms) through microelectrodes placed in the vicinity of the proximal dendrites of PCs from which recordings were obtained. Muscimol-evoked currents, like postsynaptic pair-evoked and mIPSC were recorded with 3–5 MΩ electrodes filled with the solution described above.

Unitary IPSCs were obtained from paired recordings of synaptically connected interneuron-pyramidal cell pairs. The presynaptic interneuron was recorded in current clamp, stimulated with five current injections (each 3 ms-long) at 50 ms inter-spike delay. The amplitude of the current injection was adjusted above the action potential threshold as to generate a single action potential for monitoring the corresponding synaptic current. The internal solution for presynaptic interneurons contained (mM): 120 K gluconate, 3.5 KCl, 10 HEPES, 3 ATPMg, and 0.3 $GTPNa_2$ (pH 7.2 with KOH). The intracellular solution for the postsynaptic neurons contained (mM): 100 CsCl, 5 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid K (BAPTA-K), 1 lidocaine *N*-ethyl bromide (QX314), 1 $MgCl_2$, 10 N-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES), 4 glutathione, 3 ATPMg, 0.3 $GTPNa_2$, 8 biocytin, 20 phosphocreatine. Both intracellular recording solutions were titrated to pH 7.2 and had an osmolarity ≈ 270 mOsm. The holding voltage was not corrected for the junction potential (< 4 mV). Experiments were performed in the presence of the AMPA- and NMDA-receptor blockers DNQX (10 μM) and kynurenate (2 mM) to block glutamatergic currents.

Drug application and analysis

All drugs were purchased from Sigma (St. Louis, MO), TOCRIS (Ellisville, MO). After recording an initial baseline for 10–15 min, drugs were bath-applied for 10 min or longer, until reaching a stable condition (as defined below in Statistical Analysis). NE, isoproterenol, clonidine, and phenylephrine were prepared immediately before experiments, and their exposure to light was avoided to prevent oxidation.

Data analysis and statistics

We defined a statistically stable period as a time interval (5–8 min) along which the IPSC mean amplitude measured during any 1-min assessment did not vary according to U-Mann Whitney test. All data are expressed as mean ± SE. In the analysis of mIPSCs, only well-isolated minis were considered for kinetic analysis. Recordings for the detection of mIPSC were prefiltered off-line at 2 KHz. Miniature events were then analyzed with the MiniAnalysis program (Synaptosoft, Decatur, GA) using a semiautomatic procedure. Amplitude threshold was set at ∼2σ noise, where σ noise was measured during periods of no visually detectable events and was usually <3 pA.

Mann-Whitney U-test was used to assess the effects of drug application whereas ANOVA was used for comparisons between subjects. Kolmogorov-Smirnoff test was used for comparing control with treatment for mIPSCs amplitude and frequency. Data were reported as different only if $P < 0.05$ unless indicated otherwise. Single and double asterisks indicate, respectively, $P < 0.05$ and $P < 0.01$.

Immunohistochemistry

Brains were extracted from two rats previously anesthetized using 30% isofluorane and perfused transcardially with 0.1 M PBS, followed by 4% paraformaldehyde fixative. Once removed from the skull, brains were fixed overnight at 4°C in the same solution and stored at the same temperature in cryoprotective solution (20% glycerol) until sectioning. Forty-micrometer-thick sections were cut on a microtome (Micron HM 430) and stored at 4°C in 0.1 M PBS with 0.001% NaN_3. Free-floating sections were washed in 0.1 M PBS and incubated for 1 h at RT in blocking buffer (0.1 M PBS, 3% normal goat serum (NGS), and 0.3% Triton X-100). Primary antibodies were diluted in Dilution Buffer containing 1× PBS, 3% NGS and 0.3% Tween-20. Sections were incubated at 4°C on a shaker with monoclonal mouse antiparvalbumin (1:8000; P3088 Sigma) and one of the following antibodies: α_2A Adrenergic Receptors: Polyclonal rabbit anti-α_2AAR (1:50; PC161 Calbiochem, San Diego, CA); α_2C Adrenergic Receptors: Polyclonal rabbit anti-α_2CAR (1:50; PA1-4518 Affinity BioReagents, Dublin, OH); β_2 Adrenergic Receptors: Polyclonal rabbit anti-β2AR (1:100; sc-569 Santa Cruz, Santa Cruz, CA). After primary incubation, sections were washed 5 × 10 min with 1× PBS and then incubated for 2 h at RT with secondary antibodies (Cy-5 Goat Anti-Mouse and Cy-2 Goat Anti-Rabbit—Jackson ImmunoResearch) diluted 1:200 in 1× PBS. After secondary incubation, sections were washed 5 × 10 min with 1× PBS and mounted on glass slides with DPX (Fluka 44581) and observed using a confocal microscope.

RESULTS

Effect of NE on mIPSCs and GABA unitary release probability

We first examined the effect of bath application of NE (20 μM, 10 min or more) on mIPSC in the presence of the Na^+-channel blocker TTX (0.5 μM). We found that NE increased the frequency of mIPSCs

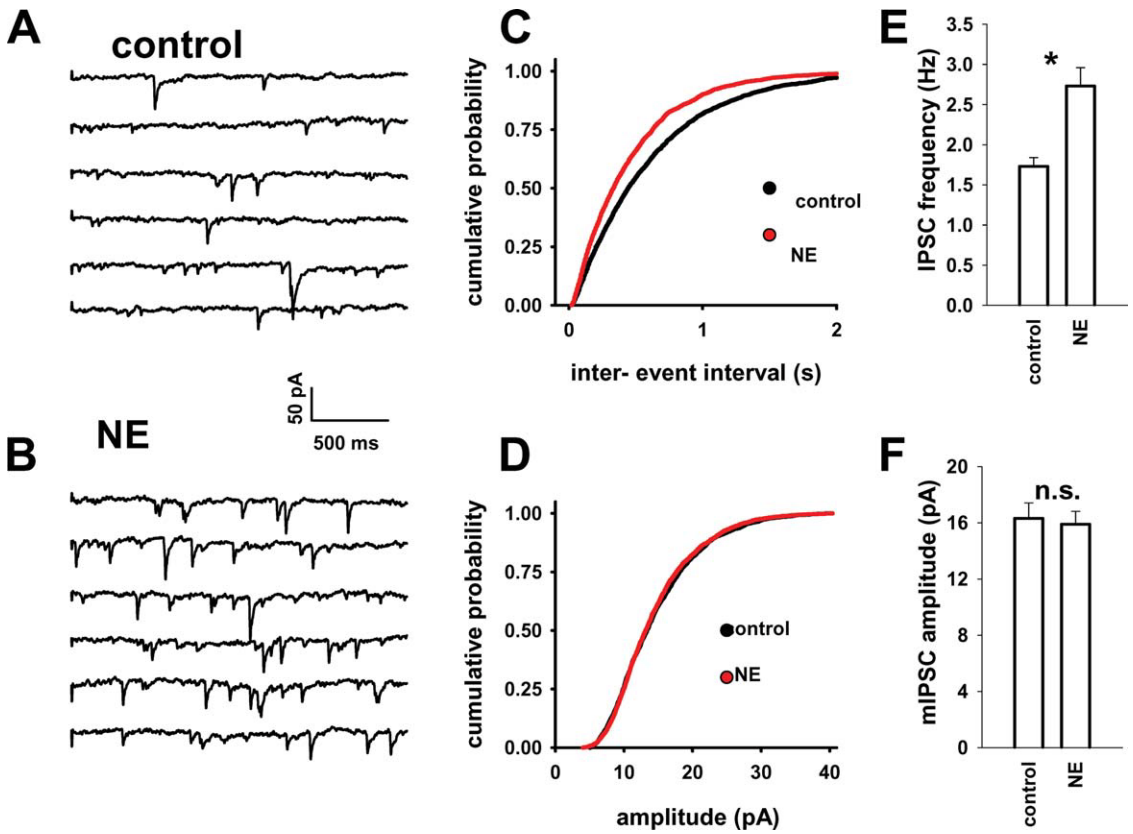

Fig. 1. NE increases the frequency but not the amplitude of mIPSCs. **A** and **B**: Representative traces illustrating mIPSCs recorded in presence of TTX (1 μM) in the absence (A) or in the presence (B) of NE (20 μM). NE increases the frequency but not mIPSC amplitude. **C** and **D**: Cumulative probability distribution of mIPSCs inter-event interval (C) and amplitude (D) before and during application of NE. E and F: effect of NE on mean mIPSC frequency and amplitude, respectively. NE increases mIPSC frequency but does not change mIPSC amplitude (the asterisk (*) indicates statistically significant differences between control and NE; Kolmogorov-Smirnoff test, $n = 15$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

from 1.73 ± 0.11 Hz (baseline) to 2.76 ± 0.22 Hz ($n = 10$, $P < 0.05$) without changing mIPSC amplitude. Figures 1A and 1B display representative traces of mIPSC in control or 15 min after bath application of NE, respectively. Figures 1C and 1D show the cumulative distribution for mIPSC inter-event interval and amplitude, respectively. A left shift in Figure 1C indicates a frequency increase, while the absence of change in Figure 1D indicates that bath-applied NE does not affect mIPSC amplitude. The effect of NE on the mean mIPSC frequency and amplitude are reported in Figures 1E and 1F, respectively. These results are consistent with a presynaptic facilitation of GABA release by NE.

An independent assessment of the effect of NE on IPSC was obtained by dual patch-clamp recordings of unitary IPSC (uIPSC). Three out of 92 cell pairs tested were synaptically connected couples interneuron –> pyramidal cell as assessed by the post hoc block of the unitary synaptic current by the $GABA_A$ receptor blocker gabazine (20 μM). Bath application of NE increased the uIPSC release probability (mean ± S.E.M.: 0.25 ± 0.05 Hz in control, Fig. 2A, vs. 0.41 ± 0.04 in NE, $n = 3$, $P < 0.05$, Fig. 2B) whereby reducing the percentage of failures. As a result, the overall uIPSC amplitude was enhanced after NE, due to the increased number of nonfailure events (uIPSC amplitude 2.3 ± 1 pA in control vs. 6.1 ± 1.2 pA in NE, $n = 3$, $P < 0.05$ Fig. 2C), while no changes were observed in the nonfailure uIPSC amplitude (10.1 ± 1.7 pA in control vs. 12.4 ± 1.6 pA in NE, $n = 3$, $P < 0.05$, Fig. 2D). Together, these data indicate a presynaptic locus for the regulation of local GABAergic synaptic transmission by NE in the auditory cortex.

Role of presynaptic α_2 and β adrenoceptors in the regulation of local GABAergic synapses

Based on our previous study (Salgado et al., 2011b), we hypothesized that the presynaptic regulation of local GABAergic transmission by NE was mediated by α_2 and/or β adrenoceptors. We therefore examined the effects of clonidine (α_2 agonist, 1 μM; representative recordings in Figs. 3A and 3B, control and treatment, respectively) and isoproterenol (β agonist,

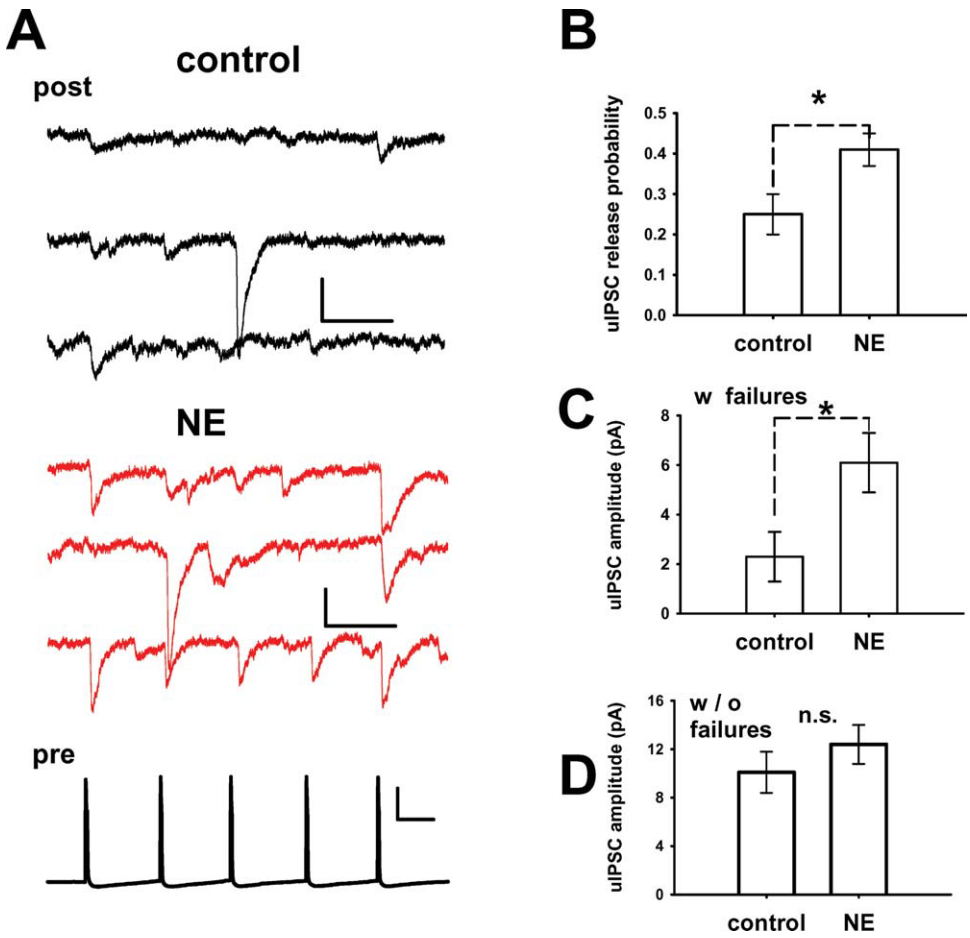

Fig. 2. NE increases GABA release probability. **A**: Upper traces represent uIPSC recorded in a postsynaptic cell (post, first three in control, next three after application of 20 μM NE) following a train of 5 action potentials (pre, lowest trace) produced in a GABAergic IN by current injections of depolarizing current (3 ms, 400 pA). Scaling; voltage clamp: 25 pA, 50 ms; current clamp: 25 mV, 50 ms. **B**: mean uIPSC frequency, calculated as [total number of evoked nonfailure synaptic events]/[total number of evoked presynaptic spikes] (n = 3). **C** and **D**: average uIPSC amplitude calculated (C) including failures (absence of synaptic event following a presynaptic spike), or excluding failures (D, same sample as B). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

50 μM; representative recordings in Figs. 3C and 3D, as above) on the frequency and amplitude of mIPSC. We found that neither clonidine nor isoproterenol changed the mIPSC amplitude (15.8 ± 0.5 pA before clonidine vs. 16.3 ± 0.4 pA after 10 min bath-application of clonidine, n = 6, P = 0.37). Similarly, bath application of isoproterenol did not significantly change the mean mIPSC amplitude (14.0 ± 0.4 pA in control vs. 14.4 ± 0.4 in isoproterenol; n = 6, P = 0.67, Fig. 3E). On the contrary, bath application of any of these agonists significantly increased mIPSC frequency from 1.37 ± 0.12 to 1.92 ± 0.17 after clonidine (n = 6, $P < 0.05$), and from 1.17 ± 0.20 to 1.86 ± 0.14 Hz following isoproterenol (n = 6 each, $P < 0.05$, Fig. 3F). Altogether, these results suggest the presence of presynaptic α_2- and β-adrenoceptors in GABAergic neurons projecting to L2/3 pyramidal neurons of the auditory cortex.

Identification of α_2- and β- adrenoceptors in parvalbumin-positive interneurons

Several types of GABAergic neurons have been identified and characterized throughout all cortical layers. An important class of cortical GABAergic cells is represented by fast-spiking interneurons (INs) containing the Ca^{2+}-binding protein parvalbumin (PV), including basket cells, whose axons project to and wrap, somata and proximal dendrites of local pyramidal neurons, as well as chandelier cells, which project to the initial axon segment of pyramidal cells (Freund, 2003). We tested the possibility that PV-positive (PV+) adrenoceptor-containing INs mediate the presynaptic increase in GABAergic signaling by studying the possible colocalization of PV with different types of adrenoceptors. We used 40-μm-thick coronal sections from the auditory cortex three rat brains to measure the degree of co-localization of PV with

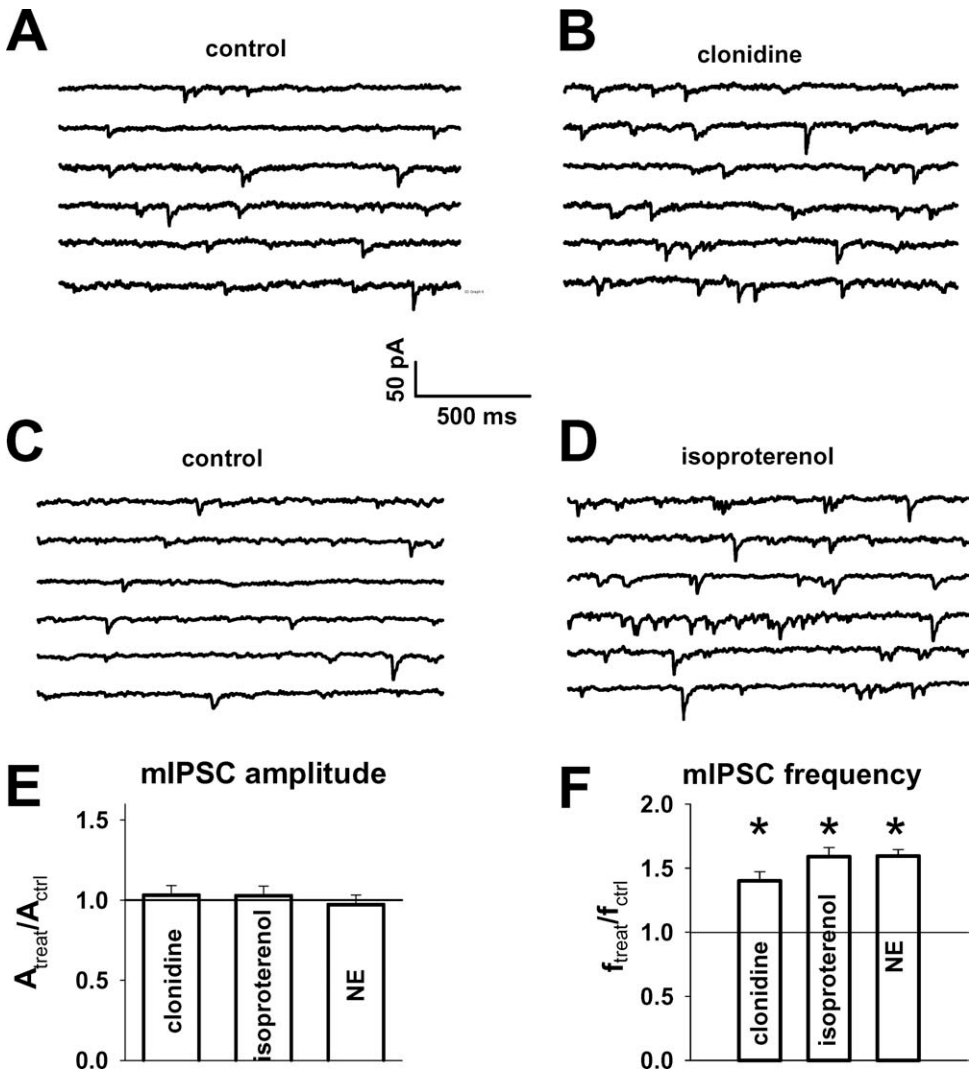

Fig. 3. Different effects of adrenoceptors activation on mIPSC properties. **A** and **B**: Representative traces displaying the effect of the α_2-adrenergic agonists clonidine (1 μM, A: control, B clonidine) on mIPSC in the presence of the Na^+-channel blocker TTX (1 μM). **C** and **D**: as above, for the β-agonist isoproterenol (50 μM, C: control, D: isoproterenol). E and F summarize the results and compare the effects of clonidine and isoproterenol with those of NE (20 μM, $n = 10$) on the mIPSC amplitude and frequency, respectively. Asterisks (*) indicate significant differences between treatment and control. Both clonidine and isoproterenol significantly increased the mIPSCs mean frequency without altering the amplitude, suggesting the presence of α_2 and β adrenoceptors on GABAergic cells projecting locally to cortical layer 2/3.

α_{2a}, α_{2b}, α_{2c}, β_1, or β_2 adrenoceptors. α_{2a}, α_{2c}, and β_2 receptors showed consistent cellular stain. A large proportion of PV+ cells colocalized with the antibodies to the selected adrenoceptors: 56% of PV+ cells colocalized with α_{2a} fluorescence, 44% of PV+ cells colocalized with α_{2c}+ cells, and 62% of PV+ cells colocalized with β_2+ cells (Figs. 4A–4C). These results, together with the pharmacology of mIPSCs, support the hypothesis that α_2 and β adrenoceptors are present in PV+ GABAergic INs in the auditory cortex.

NE-induced attenuation of postsynaptic GABAergic currents is mediated by α_1 adrenoceptors

The presence of presynaptic adrenoceptors does not rule out the possibility that NE also targets GABAergic transmission postsynaptically. We tested whether NE directly exerted any postsynaptic action on $GABA_AR$-mediated currents by assessing the effect of bath applications of NE (20 μM) on pressure-evoked responses to the $GABA_AR$ agonist muscimol (100 μM, pulse duration: 5–15 ms, evoked every 30 s). NE application reversibly decreased the amplitude of the response to muscimol by 41 ± 5 % (baseline: 405.9 ± 80 pA; NE: 238 ± 50.6 pA, $n = 6$, Fig. 5A), in contrast with saline application during a similar period, which did not evoke a significant decrease in the muscimol-evoked response ($n = 5$, $P < 0.05$, Mann-Whitney U-test, Fig. 5B). Based on our recent study (Salgado et al., 2011b), we hypothesized that the postsynaptic attenuation of IPSC by NE was mediated by activation of the α_1 adrenoceptors. To directly test

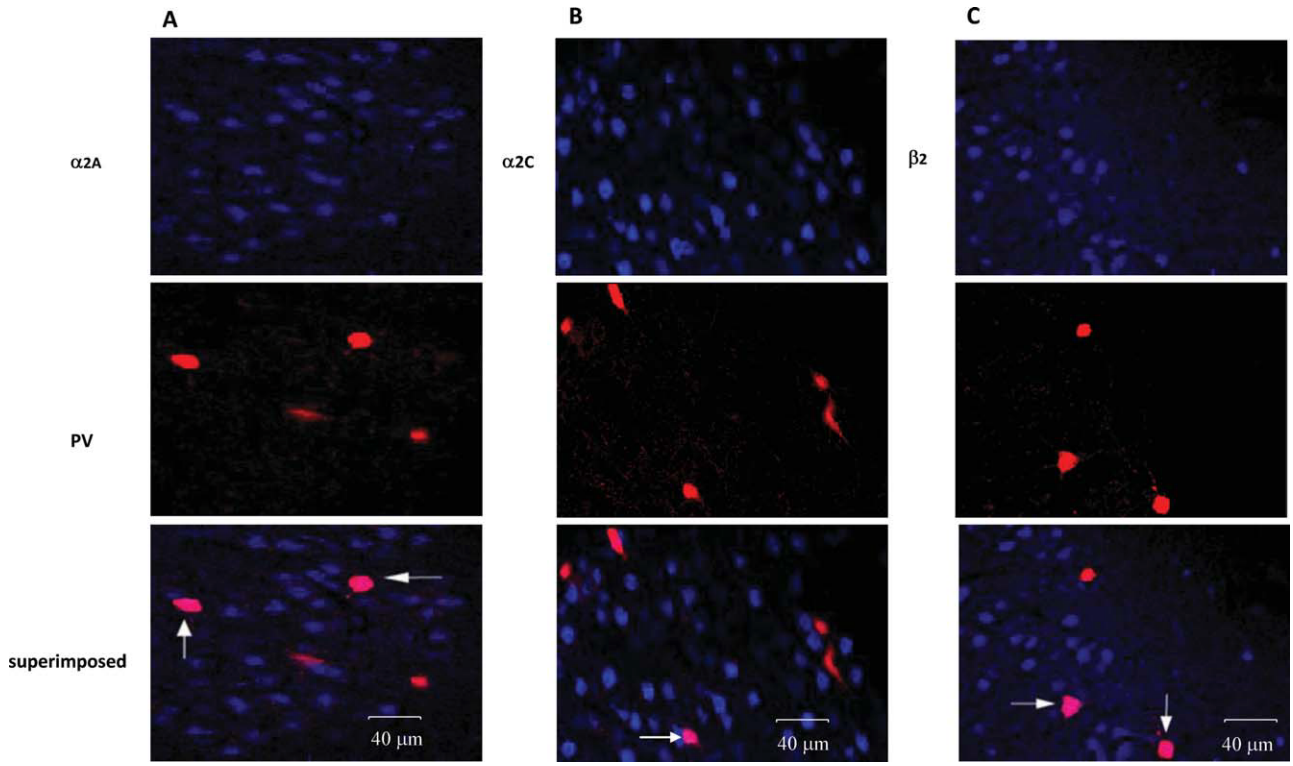

Fig. 4. α_2 and β adrenoceptors co-localize with PV-positive cells. **A**: co-localization of α_{2a} receptors with the GABAergic neuron marker PV. The region of interest (ROI) lies on the upper cortical layer 2 (the edge with cortical layer 1 is on the right of the ROI). The upper panel shows in blue cells positive for the α_{2a} adrenoceptors. The middle panel shows PV+ cells in red in the same field. Lower panel is the superimposition of the two images. Cells presenting simultaneously the two proteins result in light purple (arrows). Notice the presence of cells positive for either protein but negative the other one. **B**: same as above but for α_{2c} adrenoceptors. ROI is on upper layer 3 (layer 1 and neuropil above the ROI). C: same as A but for β_2 adrenoceptors. ROI is in cortical layer 2 (layer 1 and neuropil on the top right side). The immunochemical co-localization of PV with adrenoceptors supports a role for NE in GABAergic fast-spiking INs. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

this possibility, we first determined the effect of the α_1 agonist phenylephrine (1 μM) on muscimol-evoked response. We found that bath application of phenylephrine significantly attenuated the amplitude of the muscimol-evoked response by 44 ± 9 % (192 ± 37 pA in control vs. 109 ± 18 in phenylephrine, $n = 5$, $P < 0.05$, Mann-Whitney U-test, Fig. 5C), an effect that resembles that obtained with NE. The results on the modulation of the muscimol-evoked currents are summarized in Figure 5D.

We also examined the effect of NE in the presence of the α_1 receptor antagonist prazosin (10 μM) and of the α_1 receptor agonist phenylephrine on mIPSC amplitude. Consistent with the effect of phenylephrine on the muscimol-evoked response, we observed that NE did not change the mIPSC amplitude (19.6 ± 2.2 pA in control vs. 21.1 ± 3.5 pA in NE, n.s., *t*-Student test, $n = 8$), while phenylephrine did decrease the mIPSC amplitude from a baseline of 19.2 ± 1.1 pA to 15.8 ± 0.7 pA ($n = 10$, $P < 0.05$, Kolmogoroff-Smirnoff test) without affecting the mIPSC frequency (0.85 ± 0.18 Hz in control vs. 0.83 ± 0.17 Hz in phenylephrine, $n = 13$, n.s., Kolmogoroff-Smirnoff test). Altogether, these data support the hypothesis that activation of postsynaptic α_1 adrenoceptors is responsible for down-regulating local GABAergic signal onto layer 2/3 pyramidal neurons in the auditory cortex. Using immunohistochemical methods similar to those used to identify α_2 and β adrenoceptors, we were unable to determine the presence of α_1 adrenoceptors.

DISCUSSION

In this study, we uncovered a mechanism for enhancing cortical inhibitory transmission by NE through a presynaptic facilitation of GABA release via activation of α_2 and/or β adrenoceptors. Interestingly, such positive modulation coexists with a postsynaptic down-regulation of GABAergic inputs by α_1 adrenoceptors. To our knowledge, this is the first demonstration of two separate and opposing adrenergic mechanisms regulating local GABAergic signaling in the temporal cortex.

The pharmacology of the presynaptic increase in GABAergic function detected with electrophysiological methods is not only consistent with the immunochem-

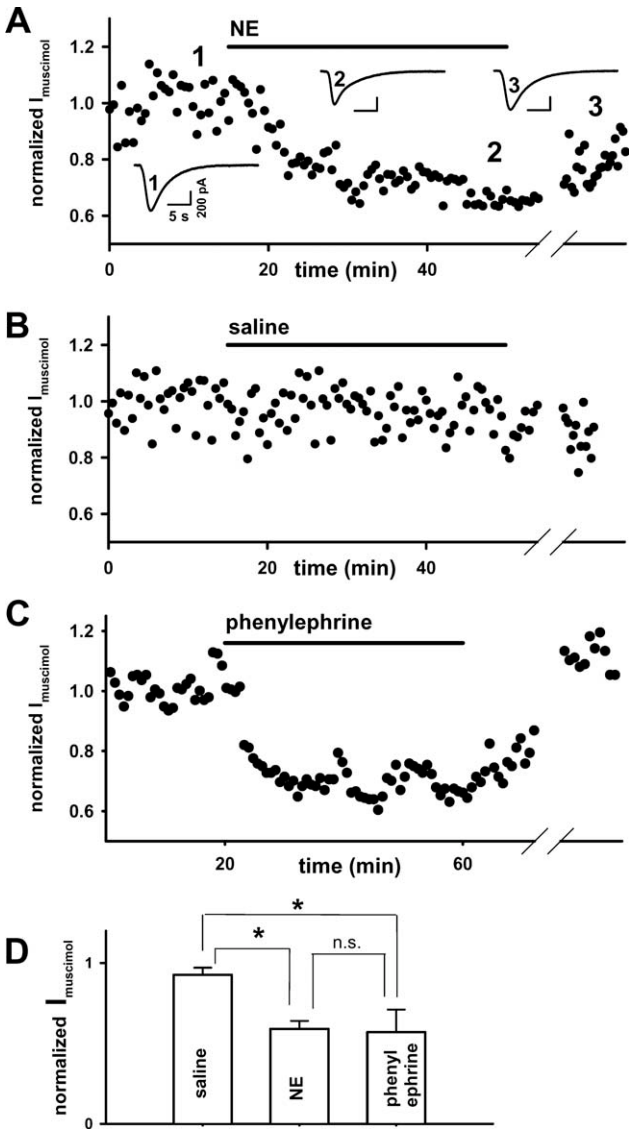

Fig. 5. NE decreases muscimol-evoked currents. **A**: Example of time course of the effect of NE (20 μM) on the inward currents produced by the application of the $GABA_AR$ agonist muscimol (100 μM). Inserts in A show traces corresponding to the points 1, 2, and 3 of the time course (control, NE, and wash, respectively). **B**: control experiment showing the effect of control saline application. The effect of NE shows as an abrupt derivative change, and is significantly larger than the modest run-down associated with saline application. **C**: Bath-application of the α_1 adrenoceptor phenylephrine (1 μM). D: Mean effect of the application of saline, NE, or phenylephrine on the muscimol-evoked current amplitude. Both NE and phenylephrine significantly decrease the muscimol-evoked current of almost 50% of the control value. The asterisks (*) or n.s. indicate the significance of statistical differences (or the absence thereof) between connected bars.

ical colocalization of PV+ with α_2- and β-adrenoceptors but is also supported by a number of previous studies reporting NE-induced increases in GABAergic function. Accordingly, two classes of pro-GABAergic adrenergic mechanisms have been described: increase in single cell excitability as found in the spinal cord (Gassner et al., 2009) and in the amygdala (Kaneko et al., 2008), possibly caused by inhibition of G-protein activated inward currents (Kobayashi et al., 2010), and synaptic mechanisms described in the cerebellum (Herold et al., 2005), bed nucleus of stria terminalis (Dumont and Williams, 2004), prefrontal cortex (Kawaguchi and Shindou, 1998), entorhinal cortex (Lei et al., 2007), or the hippocampus (Nishikawa et al., 2005).

In contrast, a number of apparently contrasting studies have reported that α_1 adrenoceptors activate the phospholipase C/protein kinase C second messenger cascade, in turn either increasing or decreasing $GABA_AR$-mediated currents, as reviewed recently (Song and Messing, 2005). In our preparation, the activation of α_1 receptors produced a postsynaptic decrease of muscimol-activated currents, most likely through the same PLC/PKC activation (Salgado et al., 2011b), although in a spiral ganglion preparation activation of α_2 receptors has also been shown to induce depression of postsynaptic currents (Zha et al., 2007).

Although the phenylephrine-induced decrease of mIPSC amplitude suggests that NE directly decreases synaptic $GABA_AR$-mediated current, the discrepancy between the effects of NE on mIPSC amplitude and the muscimol-evoked signal could be due to an overwhelming effect of NE increasing the release of GABA obscuring a decrease in mIPSC amplitude. This interpretation is further supported by the failure of NE to decrease mIPSC amplitude, in the presence of the α_1 blocker prazosin. Although the main finding of our study clearly indicates that GABAergic INs are an important target of NE in the auditory cortex, the physiological significance of the phenomenon is still elusive. In general, adrenoceptors activation enhances sensory function by increasing sensory responses (Waterhouse et al., 1998), frequency selectivity (Manunta and Edeline, 1997, 1999), and possibly by shortening the latency and lowering the detection threshold of sensory events (Drouin et al., 2007). It is a puzzling finding that NE decreases GABAergic signaling through postsynaptic activation of α_1 adrenoceptors, while at the same time increasing the release of GABA using presynaptic mechanisms. As the α_1 receptor-mediated decrease in GABAergic signaling is shared probably by most or even all GABAergic synapses onto pyramidal cells (Salgado et al., 2011b), such a widespread decrease in inhibition might simply decrease the threshold for the activation of pyramidal neurons by cortico-cortical or thalamocortical afferent, thus increasing alertness. On the other hand, the large (60–70%) increase in pyramidal cells somatic inhibition caused by increase in presynaptic release of GABA might be a powerful mechanism to enhance the production of IN-synchronized γ-oscillations, associated with enhanced attention and perception similar to what found in the olfactory system (Gire and Schoppa, 2008; Pandipati et al., 2010).

While the physiological significance of the simultaneous presynaptic increase and postsynaptic decrease in GABAergic synaptic strength awaits further investigation, it is tempting to speculate that cortical NE might exert a dual function of decreasing the activation threshold associated with alertness, while at the same time enhancing perception and sensory binding by facilitating lateral inhibition, as well as the production of γ-oscillations, through a selective enhancement of perisomatic inhibition.

ACKNOWLEDGMENTS

The authors thank Dr. Larry Cauller for critical review of the article.

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