Adrenalectomy Potentiates Noradrenergic Suppression of GABAergic Transmission in Parvocellular Neurosecretory Neurons of Hypothalamic Paraventricular Nucleus

Jian Hua Yang, Long Hua Li, Seung Yub Shin, Sora Lee, So Yeong Lee, Seong Kyu Han, and Pan Dong Ryu

1Laboratory of Veterinary Pharmacology, College of Veterinary Medicine and BK21 Program for Veterinary Science, Seoul National University, Seoul; and 2Department of Oral Physiology and Institute of Oral Bioscience, School of Dentistry, Chonbuk National University, Jeonju, Republic of Korea

Submitted 22 May 2007; accepted in final form 17 November 2007

Yang JH, Li LH, Shin SY, Lee S, Lee SY, Han SK, Ryu PD. Adrenalectomy potentiates noradrenergic suppression of GABAergic transmission in parvocellular neurosecretory neurons of hypothalamic paraventricular nucleus. J Neurophysiol 99: 514–523, 2008. First published November 21, 2007; doi:10.1152/jn.00568.2007. Glucocorticoids are known to regulate both the noradrenergic and GABAergic inputs to the paraventricular nucleus (PVN). However, little is known about the effects of glucocorticoids on the interaction of these two input systems. Here we examined the effects of bilateral adrenalectomy (ADX) on the noradrenergic modulation of GABAergic transmission in the type II PVN neurons labeled with a retrograde dye injected into the pituitary stalk. Noradrenaline either reduced or augmented the frequency of spontaneous inhibitory postsynaptic current (sIPSC) without changing the amplitude and decay time constant. These effects were blocked by α2A- and α1A/1L-adrenoceptor antagonists, respectively. ADX increased the proportion of the neurons showing the noradrenergic reduction and the extent of reduction in the IPSC frequency. The ADX-induced changes were reversed by supplementation of ADX rats with corticosterone (10-mg pellet). ADX also potentiated the noradrenergic reduction in the frequency of miniature IPSC and paired-pulse facilitation of evoked IPSC. BRL 44408 (3 μM), a α2A-adrenoceptor antagonist, blocked the noradrenergic reduction in ADX rats. Corticotropin-releasing hormone and/or vasopressin transcripts were detected in neurons displaying noradrenergic augmentation or reduction of IPSC frequency. ADX enhanced the proportion of neurons expressing corticotropin-releasing hormone. Collectively, the results suggest that depletion of corticosterone by ADX markedly potentiates the noradrenergic suppression of GABAergic transmission mediated by the α2A-adrenoceptors on the GABAergic terminals in the parvocellular neurosecretory PVN neurons. These results may provide a novel synaptic mechanism for the glucocorticoid-induced plasticity in the noradrenergic modulation of neuroendocrine function of the PVN.

INTRODUCTION

Glucocorticoids exert their feedback effects not only by inhibiting the secretion of corticotropin-releasing hormone (CRH) and vasopressin (VP) in the hypothalamic paraventricular nucleus (PVN; Jacobson 2005; Keller-Wood and Dallman 1984; Whitnall 1993) but also by modulating neuronal inputs to the PVN in the central stress pathways (Herman et al. 2003). An important target of the glucocorticoid action is the catecholaminergic pathways from the brain stem that provide the major excitatory inputs to the PVN. The A2/C2 catecholaminergic cells in the area of the solitary tract nucleus preferentially innervate the neurons in the medial paravascular zone and the A1/C1 cells in the rostral ventrolateral medulla innervate the paravascular preautonomic neurons (Cunningham and Sawchenko 1988; Cunningham et al. 1990). These pathways are activated by stress (Pacak et al. 1995) and mediate the release of CRH and adrenocorticotropin through the α1-adrenoceptors (Plotksy et al. 1989; Szafarczyk et al. 1987). On the other hand, adrenalectomy (ADX) or removal of circulating corticosterone in the rat increases the stress-induced release of noradrenaline (Pacak et al. 1995) and both α2A- (Feuvrier et al. 1999) and α1B-adrenoceptor mRNA levels in the PVN (Day et al. 2002), whereas chronic stress reduces the α2A-adrenoceptor mRNA levels in selective brain regions (Meyer et al. 2000).

Another important target of glucocorticoid action is the GABAergic inputs to the PVN. The GABAergic terminals in the PVN are abundant (Decavel and van den Pol 1990) and originate mainly from the extranuclear regions, particularly the peri-PVN areas (Boudaba et al. 1996; Herman et al. 2003; Roland and Sawchenko 1993). These GABAergic inputs are considered as local relay not only for the inhibitory inputs from the hippocampus and the prefrontal cortex but also for the excitatory signals from the amygdala to the PVN (Herman and Cullinan 1997). ADX increases the number of GABAergic synapses in the CRH neurons by 55% (Miklos and Kovacs 2002) and the frequency of inhibitory postsynaptic currents (IPSCs) in the PVN neurons (Verkuyl and Joels 2003). Stress also regulates the expression of glutamate decarboxylase mRNA (Bowers et al. 1998) and γ-aminobutyric acid type A [GABA(A)] receptors (Cullinan and Wolfe 2000) and suppresses the frequency of IPSCs in the PVN (Verkuyl et al. 2005).

Noradrenaline increases the frequency of IPSCs by activating the α1-adrenoceptors on the soma, but decreases their frequency by activating the α2-adrenoceptors (Chong et al. 2004; Daftary et al. 2000; Han et al. 2002; Li et al. 2005) and α1-adrenoceptors on the terminal of GABAergic neurons.
(Chen et al. 2006). These receptors were further identified as α1A/IL and α2A subtypes, respectively (Chong et al. 2004). Despite the fact that glucocorticoids are known to regulate both the GABAergic and the noradrenergic inputs in the PVN, whether glucocorticoids affect the noradrenergic modulation of GABAergic transmission is largely unknown. To answer these questions, we examined the effects of noradrenaline on IPSCs recorded from the putative parvocellular neurosecretory PVN neurons in the brain slices of the sham-operated and adrenalectomized rats. Putative parvocellular neurosecretory neurons were identified based on retrograde staining (Makarenko et al. 2001; Yang et al. 2007) and electrophysiological criteria (Luther et al. 2002).

METHODS

Adrenalectomy and corticosterone analysis

Male Sprague–Dawley rats (3–5 wk old; Samtaco, Seoul, Korea) were either bilaterally adrenalectomized (ADX group) via a dorsal approach, operated but left adrenal intact (sham-operated group), or adrenalectomized and supplemented with corticosterone (ADX-CS group) under 2% xyline and 5% ketamine [1:3, 2 ml/kg, administered intraperitoneally (ip)] anesthesia. For the rats in the ADX-CS group, a pellet of slow-release corticosterone (10 mg) was implanted subcutaneously to the dorsal neck region (Innovative Research of America, Sarasota, FL). The experiments were carried out in accordance with the guidelines of the Laboratory Animal Care Advisory Committee of Seoul National University. The animals were housed two to three per cage under a constant temperature and humidity, on a 12-h light/dark cycle (lights on at 8:00 am), with unrestricted access to food and water or isotonic saline (0.9%)/5% sucrose (ADX rats). Trunk blood was collected at the time of brain slice preparation (10:00 –14:00 h) 7–9 days after the ADX. Plasma was separated and transferred to a recording chamber (0.7 ml) and fixed with a grid of nylon stockings threads supported by a U-shaped silver wire weight while being perfused (2 ml/min) with oxygenated (95% O2-5% CO2) ACSF at 20°C for analysis of corticosterone. The corticosterone levels were determined by a radioimmunoassay (125I) Corticosterone Kit, MP Biomedicals, Orangeburg, NY) using a gamma counter (Wallac 1470 Wizard, Turku, Finland). The lower limit of the assay sensitivity for corticosterone was 0.7 μg/dl.

Retrograde staining and slice preparation

To label the neurosecretory PVN neurons, we anesthetized the rats with 2% xyline and 5% ketamine [1:3, 2 ml/kg, ip], fixed to a customized hypophysectomy instrument that allows access to the pituitary through the ears. We injected a fluorescent dye, 1,1′-dioctadecyl-3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI, 0.3 μl of 3% solution; Molecular Probes, Eugene, OR), to the pituitary stalk of the rats over a 1-min period using a 5-μl 3% solution (Molecular Probes, Eugene, OR), to the pituitary stalk sensitivity for corticosterone was 0.7 μg/dl. Molecular Probes, Eugene, OR), to the pituitary stalk sensitivity for corticosterone was 0.7 μg/dl. To label the neurosecretory PVN neurons, we anesthetized the rats with 2% xyline and 5% ketamine [1:3, 2 ml/kg, ip], fixed to a customized hypophysectomy instrument that allows access to the pituitary through the ears. We injected a fluorescent dye, 1,1′-dioctadecyl-3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI, 0.3 μl of 3% solution; Molecular Probes, Eugene, OR), to the pituitary stalk of the rats over a 1-min period using a 5-μl 3% solution (Molecular Probes, Eugene, OR), to the pituitary stalk sensitivity for corticosterone was 0.7 μg/dl.

Electrophysiological recording

The whole cell currents were recorded from the PVN neurons in the coronal hypothalamic slices with the visualization of individual neurons using fluorescence microscopy with a “green” filter cube (U-MWG, Olympus, Tokyo, Japan), as reported previously (Han et al. 2002). Pipettes were pulled from borosilicate glass capillaries with a 1.7-mm diameter and a 0.5-mm wall thickness. Their open resistances ranged from 2 to 5 MΩ and the seal resistances ranged from 1 to 10 GΩ. The patch pipettes were filled with a solution containing (in mM) 140 KCl, 20 HEPES, 0.5 CaCl2, 5 EGTA, and 5 MgATP (pH adjusted with KOH to 7.2). One of the slices in the incubation chamber was transferred to a recording chamber (0.7 ml) and fixed with a grid of nylon stockings threads supported by a U-shaped silver wire weight while being perfused (2 ml/min) with oxygenated (95% O2-5% CO2) ACSF at 30–33°C. Individual neurons were identified using an upright microscope with differential interference contrast (BX50WI, Olympus) for the whole cell patch recording. Electrical signals were recorded by an Axoclamp 2B amplifier (probe gain, >0.01 MU with HS-2 probe) or an Axopatch 200B. Current or voltage signals were filtered at 1 kHz and digitized at 10 kHz using an analog-digital converter (Digidata 1200) and the pClamp program (Version 8, Axon Instruments, Foster City, CA). Resting membrane potentials were corrected for the liquid junction voltage (~4.8 mV). The membrane input resistance was obtained by relating the hyperpolarizing current pulses (about ~60 pA) to classify the cell type and the respective voltage shifts.

To determine the effect of ADX on the paired-pulse ratio (PPR) of evoked IPSCs following application of noradrenaline, electrical stimuli were applied using a bipolar tungsten electrode (World Precision Instruments, Sarasota, FL) that was placed on the region adjacent to the PVN (Li et al. 2005; Verkuyt et al. 2005). Paired stimuli (0.2 ms, 0.2–0.8 mA, and 0.2 Hz) were generated with an isolated pulse stimulator (Model 2100; A-M Systems, Carlsborg, WA) at a 100-ms interstimulus interval. The PPR was expressed as the peak amplitude ratio of the second synaptic response to the first synaptic response (P2/P1).

Identification of putative parvocellular neurosecretory neurons

DiI, a highly lipophilic fluorescent dye, was used to label PVN neurons located in both the parvocellular and magnocellular subdivisions (Fig. 1A), as reported previously (Makarenko et al. 2001; Yang et al. 2007). To identify the putative parvocellular neurosecretory PVN neurons, we initially selected a labeled neuron within the presumed parvocellular division of the slice using fluorescence (Fig. 1B) and determined the type of neuron by applying electrophysiological criteria under normal light (Fig. 1, C and D). The intranuclear location of recorded neurons was estimated based on the distance from the dorsal end of the third ventricle and shape of the translucent area of the PVN (Figs. 1E and 3C). In some neurons, we additionally measured the widths and lengths of soma before recording electrophysiological properties using a scale built within the microscope. Immediately after establishing the whole cell configuration, the type of PVN neurons was determined by applying a series of depolarizing current pulses of 250 ms with a 250-ms prepulse hyperpolarizing to approximately ~100 mV (Han et al. 2002; Tasker and Dudek 1991; Fig. 1D). Neurons showing a prominent transient outward rectification were classified as type I and those showing little rectification as type II. We considered the DiI-labeled type II PVN neurons as putative parvocellular neurosecretory neurons (Luther et al. 2002). Thus type I neurons were excluded from further analyses. Figure 1E shows the distribution of some of the labeled type II neurons in the PVN. In addition, the cells were also excluded from the analyses if they did not meet the following criteria: resting membrane potential negative to ~50 mV and spontaneous synaptic activity with an unstable frequency and amplitude during the control period.
PCR amplification was carried out using a fraction of the single-cell cDNA as a template. Reaction components were as follows: 1 μM each primer, 12.5 μl of 2 × buffer (GoTaq Green Master Mix; Promega), 1 μl of dimethyl sulfoxide, and 4 μl of the cDNA template made from the single-cell RT reaction. The thermal cycling program, set at 94 °C for 40 s, 60 °C for 40 s, and 72 °C for 1 min, consisted of 50 cycles. The primer pairs used (Bioneer, Daejeon, South Korea) were: CRH (M54987), 5'-AAC TCA GAG CCC AAG TTT TTT TTT -3' and 5'-GGA CAT GAT GCC TCA GTA GAC -3' (440 bp); and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, X02231), 5'-GGA CAT TGT TGC CAT CAA CGC -3' and 5'-ATG AGC CCT TCC ACG ATG CCA AGG -3' (245 bp).

Negative controls for contamination from the extraneous and genomic DNA were run for every batch of neurons by omitting the reverse transcriptase or replacing the cellular template with water. All the PCR products were purified using a PCR purification kit (Qiagen, Hilden, Germany) and the purified products were sequenced with gene-specific primers to confirm the amplified sequences. DNA sequencing was performed at the National Instrumentation Center for Environmental Management at Seoul National University.

RESULTS

Whole cell patch-clamp recordings were performed on 150 labeled type II PVN neurons. The labeled neurons did not express any significant low-threshold spike, which is characteristic of the neurosecretory PVN neurons (Luther et al. 2002), and expressed the mRNA of CRH and/or VP in 8 of 20 neurons tested (data not shown). The resting membrane potential and input resistance of labeled type II PVN neurons were, respectively, −58.5 ± 1.12 mV and 497 ± 30 MΩ (n = 33) in the sham-operated rats and −58 ± 1.2 mV and 529 ± 32 MΩ (n = 19) in the ADX rats. The width and length of the soma were 7.5 ± 0.22 and 17 ± 0.80 μm (n = 22) in the sham-operated rats and 7.8 ± 0.27 and 17.4 ± 0.43 μm (n = 17) in the ADX rats. These observations indicate that the Dil-labeled type II PVN neurons are likely to represent the parvocellular neurosecretory neurons in the PVN.

Noradrenergic modulation of sIPSCs in parvocellular neurosecretory PVN neurons

The labeled type II PVN neurons showed rich spontaneous (s)IPSCs at the resting state. The mean baseline frequency, amplitude, and decay time constant of the sIPSCs were determined from the 3- to 5-min segments of the current records according to the methods previously described (Han et al. 2002) using a Mini Analysis Program (Version 4.0, Synaptosoft; http://www.synaptosoft.com). The effects of noradrenaline on the frequency, amplitude, and decay time constant of IPSCs were presented relative to the corresponding control values immediately before applying the drug. All data are presented as means ± SE and the number of neurons tested and analyzed is denoted by “n.” Statistical comparisons were performed with the appropriate analyses, including χ² test, unpaired and paired Student’s t-tests, and ANOVA with post hoc Newman–Keuls test. A value of P < 0.05 was considered significant.

Single-cell reverse transcription–polymerase chain reaction (RT-PCR)

The single-cell RT-PCR was performed as described previously with minor modifications (Di et al. 2003; Glasgow et al. 1999; Yang et al. 2007). Briefly, the cytoplasm of neuron was gently aspirated under visual control into a patch-clamp recording electrode, with care taken not to aspirate the nucleus. The contents of the electrode were subsequently dissipated into a microtube containing (in μl): 2.9 of diethyl pyrocarbonate-treated water, 0.7 of bovine serum albumin, 0.7 of random hexamer (100 ng/μl), and 0.7 of RNaSeOUT (40 U/μl). After aspiration of the neuron, the microtube including cytoplasm was either stored at −70 °C or immediately used for RT. Single-strand cDNA was synthesized from the cellular mRNA using Superscript III. All reagents except random hexamer (Promega, Madison, WI) were obtained from Invitrogen (Carlsbad, CA).
in the neurons showing the noradrenergic reduction, but increased the frequency by 2.1-fold (from 2.8 ± 0.68 to 5.22 ± 1.18 Hz; P < 0.01, n = 15) in the neurons showing the noradrenergic augmentation in the sIPSC frequency. In contrast to the modulation of the sIPSC frequency, noradrenaline did not alter the amplitude or decay time constant of the sIPSCs recorded from the neuron groups showing either noradrenergic reduction or augmentation in the sIPSC frequency (data not shown). These results are consistent with previous reports on pooled parvocellular PVN neurons (Chong et al. 2004; Han et al. 2002). Based on earlier reports, we further determined whether the noradrenergic augmentation and reduction of the sIPSC frequency in neurosecretory parvocellular neurons are also mediated by α1A/1L- and α2A-adrenoceptor subtypes, respectively (Fig. 2). In 4 of 5 neurons displaying noradrenergic reduction, the effect of noradrenaline was blocked with 3 μM 2-[(4,5-dihydro-1H-imidazol-2-yl)methyl]-rsqb[2,3-dihydro-1-methyl-1H-isooindole maleate (BRL 44408), an α2A- adrenoceptor antagonist (Fig. 2F). In 5 of 6 neurons showing noradrenergic augmentation, the effect of noradrenaline was blocked with 1 μM prazosin, an α1L/1A-adrenoceptor antagonist (Fig. 2G). BRL 44408 (3 μM) did not affect the noradrenergic augmentation of sIPSC frequency (n = 3). These results collectively indicate that noradrenaline can either decrease or increase GABAergic inhibitory transmission in parvocellular neurosecretory PVN neurons via α2A- and α1L/1A- adrenoceptors, respectively.

Effects of ADX on noradrenergic modulation of sIPSCs

To study the effects of corticosterone on the noradrenergic modulation of GABAergic transmission, the circulating corticosterone was depleted by ADX or restored by implanting a corticosterone pellet after ADX (ADX-CS). The plasma corticosterone levels were 13.1 ± 2.83 μg/dl in the sham-operated rats (n = 7), below the detection limit (0.7 μg/dl) in the ADX rats (n = 13) and 3.5 ± 0.56 μg/dl in the ADX-CS rats (n = 14). The frequency of sIPSC in ADX rats was 0.19 ± 1.06 Hz, about twice that in sham-operated rats (3.2 ± 0.45 Hz, P < 0.05). The elevation of sIPSC frequency was not observed in ADX-CS rats receiving a corticosterone supplement (2.83 ± 0.66 Hz in ADX-CS rats vs. 3.2 ± 0.45 Hz in sham-operated rats, P = 0.3). ADX did not alter either the amplitude or the decay time constant (P > 0.2).

An obvious finding on the neurons from ADX rats was a significant increase in the proportion of the neurons showing the noradrenergic reduction in the sIPSC frequency. In the neurons from ADX rats, the proportion of neurons showing the noradrenergic reduction in the sIPSC frequency increased by twofold (from 46% in the sham-operated rats to 89% in ADX rats), but decreased the proportion of neurons showing the noradrenergic augmentation to one fifth (from 54% in the sham-operated rats to 8% in ADX rats). The noradrenergic reduction in the frequency of sIPSCs was, respectively, 74 ± 4.5 and 49 ± 5.3% in the neurons from ADX and sham-operated rats (P < 0.05; Fig. 3B). Such ADX-induced changes in the noradrenergic reduction were not observed in the ADX-CS rats supplemented with corticosterone (10-mg pellet). As illustrated in Fig. 3C, most of the labeled neurons examined were located in the mediodorsal...
parvocellular part of the PVN, but there was no obvious segregation between the two neuron groups showing noradrenergic augmentation and reduction of sIPSC frequency. Taken together, these results suggest that depletion of corticosterone by ADX markedly potentiates the noradrenergic suppression of GABAergic transmission in the parvocellular neurosecretory PVN neurons.

Effects of ADX on noradrenergic modulation of mIPSCs

To determine whether the noradrenergic suppression of GABAergic transmission is due to a direct effect of noradrenaline on the GABAergic terminals, we examined the effect of noradrenaline on the mIPSCs recorded in the presence of TTX (1 μM), a Na⁺ channel blocker that blocks neuronal firing (Fig. 4). In a similar manner to that observed in the sIPSC, the frequency of mIPSC in the ADX rats was significantly higher than that in the sham-operated rats (7.1 ± 1.85 Hz, n = 6 vs. 2.81 ± 0.67 Hz, n = 7; P < 0.05). However, the amplitude and decay time constant of mIPSCs were not different between the ADX and sham-operated rats (96.7 ± 15.8 vs. 100 ± 20.2 pA, 7.13 ± 0.63 vs. 7.69 ± 0.65 ms, P > 0.05).

Noradrenaline decreased the mIPSC frequency in all the type II neurons tested from both the sham-operated (n = 7) and ADX rats (n = 6). The effect of noradrenaline on the mIPSC frequency was reversible (Fig. 4, A and B) and induced a rightward shift in the cumulative frequency plots of interevent intervals of mIPSCs (Fig. 4, C and D). As in the sIPSC, the extent of reduction in the mIPSC frequency in ADX rats was also significantly larger than that in the sham-operated rats. However, noradrenaline did not affect the amplitude and decay time constant of the mIPSCs in either the sham-operated or ADX rats. On average, noradrenaline (100 μM) reduced the frequency of mIPSC by 33 ± 3.4% in the sham-operated rats (P < 0.05) and by 56 ± 7.9% in the ADX rats (P < 0.05). The extent of noradrenaline-induced reduction in the ADX rats was significantly higher than that in the sham-operated rats (33 vs. 56%, P < 0.05; Fig. 4E). Overall, these results further suggest that the noradrenergic reduction in the IPSC frequency is exerted either by a mechanism independent from TTX-sensitive action potential or by activation of the receptors located on the GABAergic terminals that are in contact with the parvocellular neurosecretory neurons in the PVN.
Effect of ADX on paired-pulse ratio of evoked IPSCs

Our earlier observation (Fig. 4) indicates that noradrenaline depresses the GABAergic transmission through a presynaptic action, either by affecting the release probability of GABA or by changing the number of functional GABAergic synapses. To further identify the presynaptic mechanism of the noradrenergic suppression of GABAergic transmission, we examined the effect of noradrenaline on the paired-pulse ratio (PPR) recorded from DiI-labeled type II PVN neurons showing noradrenergic suppression of GABAergic transmission. Prior to the application of noradrenaline, neurons showed weak paired-pulse facilitation and PPR values were similar between the sham-operated and ADX rats (1.06 ± 0.04 in sham-operated, n = 6, vs. 1.15 ± 0.03 in ADX, n = 6; P > 0.05 by ANOVA with a post hoc Newman–Keuls test). The extent of the noradrenaline-induced increase in PPR (1.45 ± 0.06 in sham-operated vs. 1.98 ± 0.11 in ADX rats; P < 0.01 by ANOVA with a post hoc Newman–Keuls test). The extent of the noradrenaline-induced increase in PPR was significantly larger in the ADX rats (172 ± 9.8%), compared with their sham-operated counterparts (137 ± 3%, P < 0.05 by Student’s t-test; Fig. 5B).

Adrenoceptors mediating noradrenergic reduction in ADX rats

Figure 6 shows that BRL 44408, a selective antagonist of α2A-adrenoceptors (pA2 = 8.0; Alexander et al. 2001) blocked the noradrenaline-induced reduction in the sIPSC frequency in the type II neurons of the ADX rats. The time course histograms of sIPSC frequency and current records before and after applying noradrenaline show that BRL 44408 (3 μM) reversibly blocked the noradrenergic reduction (Fig. 6, A and B). BRL 44408 itself did not affect the sIPSC frequency. Similar results were observed in six of six type II neurons from the ADX rats (n = 4) that showed the noradrenergic reduction in the sIPSC frequency (Fig. 6C). In these neurons, noradrenaline decreased the sIPSC frequency to 34% of that observed in the

![Figure 5](http://jn.physiology.org/)

**FIG. 5.** Effect of ADX on the noradrenaline-induced paired-pulse facilitation of evoked IPSCs. A: typical current traces showing paired-pulse responses during control and application of noradrenaline in the sham-operated and ADX rats. Stimulation artifacts were removed for clarity. B: summary data showing the relative paired-pulse ratio (PPR) in the neurons from sham-operated (n = 6) and ADX (n = 6) rats. PPR<sub>Control</sub> and PPR<sub>NA</sub> represent, respectively, the PPVs before application of noradrenaline and during application of noradrenaline. *P < 0.05 by Student’s t-test.

![Figure 6](http://jn.physiology.org/)

**FIG. 6.** Blockade of the noradrenergic reduction in the sIPSC frequency by BRL 44408, a α<sub>2A</sub>-adrenoceptor antagonist in the DiI-labeled type II PVN neurons of ADX rats. A: the time course histogram showing the noradrenaline-induced reduction in the sIPSC frequency in a neuron. B: the current traces shown by the arrows in A. C: effects of noradrenaline on the sIPSC frequency of the neurons from ADX rats in the presence or absence of BRL 44408 (3 μM, n = 4). **P < 0.01 by Student’s t-test. NA 100, noradrenaline 100 μM; BRL 3, BRL 44408 3 μM.
control (4.44 ± 1.58 Hz, \( P < 0.01 \)), but failed to reduce the sIPSC frequency in the presence of BRL 44408 (\( P = 0.772 \)). This result suggests that \( \alpha_{2A} \)-adrenoceptors also mediate the noradrenergic suppression of GABAergic transmission in the parvocellular neurosecretory PVN neurons of the ADX rats.

Expression of CRH and VP in the PVN neurons showing noradrenergic suppression of GABAergic transmission

CRH and VP from the neurosecretory parvocellular neurons are the major stimulators of the hypothalamic-pituitary-adrenal (HPA) axis. Using the single-cell RT-PCR technique (Di et al. 2003; Glasgow et al. 1999; Yang et al. 2007), we further examined the effects of ADX on the expression of these peptides in parvocellular neurosecretory PVN neurons and correlations between the effects of noradrenaline on sIPSC and peptide expression. In sham-operated rats, of the 19 neurons showing noradrenergic augmentation in sIPSC frequency, 2 expressed both peptides, 2 expressed CRH, and 3 expressed VP (Fig. 7A). Among another 19 neurons showing noradrenergic reduction in sIPSC frequency, 2 expressed both peptides, 1 expressed CRH, and 4 expressed VP, respectively (Fig. 7B). In ADX rats, of the 14 neurons showing noradrenergic reduction, 3 expressed both peptides, 2 expressed CRH, and 1 expressed VP (Fig. 7C). Our results demonstrate that expression of CRH, but not VP, is significantly enhanced in ADX rats (18%, 7 of 38 cells in sham-operated vs. 36%, 5 of 14 cells in ADX; \( P < 0.05 \) by \( \chi^2 \) test). The results also indicate that noradrenergic modulation of GABAergic transmission is not correlated with expression of CRH and/or VP in neurosecretory parvocellular PVN neurons.

**DISCUSSION**

The data described in the present study demonstrate that the depletion of the circulating glucocorticoids by ADX potentiates the noradrenergic suppression of GABAergic transmission in the parvocellular neurosecretory PVN neurons. We found that ADX increased the proportion of neurons showing the noradrenergic suppression and extent of the suppression. Furthermore, supplementing the ADX rats with corticosterone prevented these changes, suggesting that the potentiation of the noradrenergic suppression was due to the depletion of corticosterone by ADX. The results are consistent with the report showing that blocking GABAergic transmission in the PVN with bicuculline increases activity of the HPA axis (Cole and Sawchenko 2002). The findings are also in good agreement with previous reports showing an elevated neuronal activity in the parvocellular PVN neurons of the ADX rats after restraint (Fevurly and Spencer 2004; Imaki et al. 1995; Roske et al. 2002) or hypovolemia (Tanimura and Watts 2000). Since ADX also augments the stress-induced release of noradrenaline (Pacak et al. 1995), corticosterone may collaborate with the noradrenergic system in regulation of the release of PVN peptides such as CRH and VP. This idea is further supported by the recent study showing that ADX increases neuronal excitability by altering the pattern and frequency as well as the noradrenergic modulation of spontaneous action potentials in the neurosecretory parvocellular PVN neurons (Yang et al. 2007). Thus our findings provide experimental evidences for a presynaptic mechanism of catecholaminergic excitation in the regulation of the HPA axis (Pacak et al. 1995; Plotsky 1987; Szafarczyk et al. 1987) and indicate that the noradrenergic modulation of GABAergic transmission is under a tonic control of glucocorticoid.

The ADX-induced increase in the proportion of neurons showing noradrenergic reduction in IPSC frequency is likely due to up-regulation or sensitization of presynaptic \( \alpha_{2A} \)-adrenoceptors mediating the noradrenergic reduction in IPSC frequency in unidentified (Chong et al. 2004; Han et al. 2002) and presynaptic parvocellular PVN neurons (Li et al. 2005). In parvocellular neurosecretory PVN neurons, BRL 44408, but not tetrodotoxin, blocked the noradrenergic suppression of GABAergic transmission, indicating that presynaptic \( \alpha_{2A} \)-adrenoceptors mediate this process. This evidence is reinforced by the finding that ADX significantly increased the paired-pulse facilitation induced by noradrenaline and decreased the frequency of mIPSCs without changing the amplitude and decay kinetics. The data additionally suggest that noradrenaline decreases the release probability of GABA (Wilcox and Dichter 1994). In view of the finding that \( \alpha_{2A} \)-adrenoceptors or their mRNA levels are down-regulated by chronic stress or glucocorticoids (Feuvrier et al. 1999; Fluge 1999), it is likely that ADX-induced depletion of circulating corticosterone causes up-regulation of \( \alpha_{2A} \)-adrenoceptors and enhancement of tissue sensitivity to noradrenaline (Bourne and Zastrow 2004). Further studies are required to clarify whether the up-regulation of presynaptic \( \alpha_{2A} \)-adrenoceptors is associated with the increase in the number of GABAergic synapses in ADX rats (Miklos and Kovacs 2002).

In addition to \( \alpha_{2A} \)-adrenoceptors, noradrenaline can enhance the frequency of IPSCs in the PVN via \( \alpha_1 \)-adrenoceptors on the soma (Han et al. 2002; Chong et al. 2004) or reduce frequency...
via $\alpha_1$-adrenoceptors on the axonal terminal of GABAergic neurons (Chen et al. 2006). Therefore down-regulation of $\alpha_1$-adrenoceptors on the soma of GABAergic neurons (Feuvrier et al. 1999) or up-regulation of $\alpha_1$-adrenoceptors on the GABAergic terminal (Day et al. 1999) may promote the ADX-induced increase in the proportion of neurons showing noradrenergic reduction of IPSC frequency. However, it is unlikely that $\alpha_1$-adrenoceptors on the soma of presynaptic GABAergic neurons are important in mediating ADX-induced changes in the noradrenergic modulation of GABAergic transmission, since the results obtained using TTX and paired-pulse stimulation (Figs. 4 and 5) strongly suggest a significant role of presynaptic adrenoceptors. Moreover, $\alpha_1$-adrenoceptors on the GABAergic terminal do not appear to play a key role in the noradrenergic suppression of GABAergic transmission, since the reduction in sIPSC frequency was completely blocked by a $\alpha_{2A}$-adrenoceptor antagonist (Figs. 3 and 6).

Molecular mechanisms for the noradrenergic inhibition of GABAergic transmission may involve the suppression of voltage-gated Ca$^{2+}$ channels and activation of inwardly rectifying K$^+$ channels via stimulation of $\alpha_{2A}$-adrenoceptors (Bylund 1995). Another possible mechanism for potentiation of the noradrenergic suppression observed in the PVN is a change in downstream events, such as G proteins (Okuhara et al. 1997; Saito et al. 1989) that mediate the intracellular signal transduction pathways of multiple neurotransmitter systems, including $\alpha_{2A}$-adrenoceptors (Chabre et al. 1994). A third possible mechanism involves alterations in retrograde signaling systems, such as nitric oxide (Bains and Ferguson 1997; Li et al. 2002; Stern et al. 2001) and endocannabinoids (Di et al. 2003, 2005) that regulate GABAergic synaptic transmission. Further studies are required to determine whether activation of $\alpha_{2A}$-adrenoceptors can influence these messenger systems in the PVN. Our results collectively indicate that circulating corticosterone can control inhibitory inputs to parvocellular neurosecretory PVN neurons by inversely regulating $\alpha_{2A}$-adrenoceptors at the GABAergic terminals on neurosecretory parvocellular PVN neurons.

The ADX-induced increase in the baseline frequency of IPSC observed in neurosecretory parvocellular PVN neurons further confirms previous data obtained with pooled parvocellular neurons (Verkuyl and Joels 2003). This ADX-induced augmentation could be attributed to an increase in the number of GABAergic synapses or neurotransmitter release probability. Our observation that ADX does not alter the paired-pulse ratio of evoked IPSCs indicates that the release probability of GABA is not affected, supporting the theory of an increase in the number of GABAergic synapses in the ADX (Miklos and Kovacs 2002). However, the results are not consistent with the findings that ADX increases the neuronal excitability (Kasai et al. 1988; Yang et al. 2007) and the fos-like immunoreactivity in the PVN (Jacobson et al. 1990). Therefore the significance of ADX-induced augmentation in GABAergic transmission should be assessed together with the changes in other factors such as the excitatory synaptic inputs and the intrinsic properties of the PVN neurons.

Since the rat brains were examined on the 7th day after ADX, it is unlikely that the changes in the GABAergic transmission or its noradrenergic modulation were due to the removal of the fast and nongenomic effects of glucocorticoids (Di et al. 2003; Keller-Wood and Dallman 1984). Of the two types of corticosteroid receptors that mediate the classical genomic effects of glucocorticoids, corticosterone can preferentially occupy the mineralocorticoid receptors (MRs; $K_d \sim 0.5$ nM) at concentrations < 3 $\mu$g/dl, but can occupy both the MR and glucocorticoid receptors (GRs; $K_d \sim 2.5$–5 nM) at higher levels such as during stress or during the diurnal peak of pituitary activity (half-maximal occupation at 25 $\mu$g/dl; Dallman 1993; Reul and de Kloet 1985). In this study, the potentiation in noradrenergic suppression of GABAergic transmission was reversed by a low corticosterone replacement (10 mg) that maintained circulating corticosterone at about 3.5 $\mu$g/dl, at which concentration most MRs are occupied, but most GRs are unoccupied (Reul and de Kloet 1985). It has been shown that the same dose of corticosterone replacement (10 mg) reverses the ADX-induced burst firing pattern and potentiation of noradrenergic excitation (Yang et al. 2007) and the ADX-induced increase in $\alpha_{1A}$-adrenoceptors mRNA to the control in the PVN (Day et al. 1999). Recent studies also have demonstrated that PVN neurons express MRs (Han et al. 2005) and 11$\beta$-hydroxysteroid dehydrogenase type II, which rapidly converts corticosterone to an inactive metabolite, thus allows much less abundant aldosterone to bind to MRs (Zhang et al. 2006). All these highlight the significance of MRs in the PVN of ADX rats. Further studies are needed to determine the subtype of corticosteroid receptors mediating the ADX-induced changes in the PVN.

Functional significance of potentiation of noradrenergic suppression in the PVN

Previous studies showed that noradrenergic inhibition of GABAergic transmission is mediated by $\alpha_{2A}$-adrenoceptors in the pooled PVN neurons (Chong et al. 2004; Han et al. 2002) and in the preautonomic PVN neurons projecting to the spinal cord (Li et al. 2005). The present study further demonstrates that ADX potentiates the $\alpha_{2A}$-adrenoceptor–mediated noradrenergic suppression of GABAergic transmission in parvocellular neurosecretory PVN neurons and that this circuit can be a pivotal target for the action of glucocorticoid negative feedback.

It is rather unexpected that the proportions of PVN neurons showing noradrenergic reduction and augmentation in spontaneous IPSCs are not different between the pooled (Chong et al. 2004; Han et al. 2002) and neurosecretory type II PVN neurons. Furthermore, the present study reveals that neurosecretory type II PVN neurons expressing mRNA of CRH, VP, or both peptides are subject to regulation of both noradrenergic reduction and augmentation of IPSC frequency. These findings suggest that the $\alpha_{1A/II}$-adrenoceptor–mediated augmentation and $\alpha_{2A}$-adrenoceptor–mediated reduction of IPSC frequency constitute a common pattern of noradrenergic modulation in PVN neurons, regardless of the peptide phenotype and projection (Chong et al. 2004; Han et al. 2002; Li et al. 2005).

GABAergic inputs to the PVN originate from the peri-PVN region as well as hypothalamic and telencephalic nuclei, including the dorsomedial and medial preoptic nuclei and the bed nucleus of stria terminalis (Herman et al. 2003). The peri-PVN GABAergic cells relay the glutamatergic inputs from the hippocampus and cortex, resulting in suppression of the HPA responses to stressful stimuli (DiIorio et al. 1993; Figueiredo et al. 2003) as well as GABAergic inputs from the amygdala,
which increase the HPA responses by disinhibition (Swanson and Petrovich 1998). Therefore potentiation of the noradrenergic suppression of GABAergic transmission at low levels of circulating glucocorticoids such as in ADX rats would limit the inhibitory inputs from the hippocampus and cortex, but enhance the excitatory inputs from the amygdala. Further study is needed to determine whether the noradrenergic disinhibition of GABAergic transmission is diminished in the PVN at high levels of glucocorticoids such as in the rats under stress. Functionally, the circuit also provides a novel mechanism for an interaction between the brain stem noradrenergic signals for systemic/interoceptive stimuli (e.g., hypoxia, ether, or cardiovascular and immune stimuli) and the GABAergic signals for the inhibitory inputs from the limbic system nuclei for processive/exterceptive stressors (e.g., restraint, fear conditioning, or exposure to a novel environment; Dayas et al. 2001; Herman et al. 2003; Sawchenko et al. 2000).

In conclusion, we have shown that when the resting corticosterone is depleted by ADX, the noradrenergic suppression of GABAergic transmission is potentiated in the parvocellular neurosecretory PVN neurons, and that such noradrenergic suppression is due to a reduction in release probability at the axon terminals of GABAergic neurons via $\alpha_{1A}$ adrenoceptors. The results suggest that the glucocorticoids can control the excitability of the parvocellular neurosecretory neurons by collaborating with the noradrenergic system in regulation of the strength of GABAergic transmission in the PVN.

ACKNOWLEDGMENTS

The authors thank Dr. Kyungjin Kim for invaluable advice and Dr. Kiho Lee and I. H. Jo for technical assistance.

GRANTS

This study was supported by Korea Research Foundation Grant KRF-2002-015-E0020.

REFERENCES


