Noradrenaline Excites and Inhibits GABAergic Transmission in Parvocellular Neurons of Rat Hypothalamic Paraventricular Nucleus

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Received 15 October 2001; accepted in final form 8 January 2002

Han, Seong Kyu, Wonee Chong, Long Hua Li, In Se Lee, Kazuyuki Murase, and Pan Dong Ryu. Noradrenaline excites and inhibits GABAergic transmission in parvocellular neurons of rat hypothalamic paraventricular nucleus. J Neurophysiol 87: 2287–2296, 2002; 10.1152/jn.00842.2001. Noradrenaline (NA) is a major neurotransmitter that regulates many neuroendocrine and sympathetic autonomic functions of the hypothalamic paraventricular nucleus (PVN). Previously NA has been shown to increase the frequency of excitatory synaptic activity of parvocellular neurons within the PVN, but little is known about its effects on inhibitory synaptic activity. In this work, we studied the effects of NA (1–100 μM) on the spontaneous inhibitory synaptic currents (sIPSC) of type II PVN neurons in brain slices of the rat using the whole cell patch-clamp technique. Spontaneous IPSCs were observed from most type II neurons (n = 121) identified by their anatomical location within the PVN and their electrophysiological properties. Bath application of NA (100 μM) increased sIPSC frequency by 256% in 59% of the neurons. This effect was blocked by prazosin (2–20 μM), the α1-adrenoceptor antagonist and mimicked by clonidine (50 μM), the α2-adrenoceptor agonist. However, in 33% of the neurons, NA decreased sIPSC frequency by 54%, and this effect was blocked by yohimbine (2–20 μM), the α2-adrenoceptor antagonist and mimicked by clonidine (50 μM), the α2-adrenoceptor agonist. The Na+ channel blocker, tetrodotoxin (0.1 μM) blocked the α1-adrenoceptor–mediated effect, but not the α2-adrenoceptor–mediated one. Both of the stimulatory and inhibitory effects of NA on sIPSC frequency were observed in individual neurons when tested with NA alone, or both phenylephrine and clonidine. Furthermore, in most neurons that showed the stimulatory effects, the inhibitory effects of NA were unmasked after blocking the stimulatory effects by prazosin or tetrodotoxin. These data indicate that tonic GABAergic inputs to the majority of type II PVN neurons are under a dual noradrenergic modulation, the increase in sIPSC frequency via somatic or dendritic α1-adrenoceptors and the decrease in sIPSC frequency via axonal terminal α2-adrenoceptors on the presynaptic GABAergic neurons.

INTRODUCTION

Noradrenergic inputs from the brain stem are critical for the correct functioning of multiple hypothalamic neuronal networks. Various neuroendocrine networks located within the hypothalamus are known to depend on noradrenaline (NA) signals to maintain endocrine homeostasis including the networks for corticotropin-releasing hormone (CRH) (Pacak et al. 1995; Plotsky et al. 1989; Whitnall 1993), gonadotropin-releasing hormone (Herbison 1997), and oxytocin/vasopressin (Leng et al. 1999).

In the paraventricular nucleus (PVN) of the hypothalamus, NA increases the release of CRH via α1-adrenoceptors, but decreases its release via α2-adrenoceptors (Plotsky 1989). Morphologically, it is known that the parvocellular neurons in the PVN receive dense noradrenergic projections from the A2 and A6 (locus coeruleus) noradrenergic cell groups (Cunningham and Sawchenko 1988; Sawchenko and Swanson 1982). Adrenergic α1, α2, and β receptors have all been identified in the parvocellular neurons (Cummings and Seybold 1988; Little et al. 1992) and in the CRH-secreting neurons (Liposits et al. 1986). In electrophysiological studies on PVN neurons, NA induced both stimulatory and inhibitory effects (Inenaga et al. 1986; Kim et al. 1989). A recent patch-clamp study (Daftary et al. 2000) further provided possible mechanisms of NA-induced changes in the excitability of PVN neurons by showing that the noradrenergic increase in the frequency of excitatory synaptic potentials occurs through α1-adrenoceptors on local glutamatergic neurons while the noradrenergic suppression of neuronal excitability was exerted through β-adrenoceptors on the cell body.

Neurons in the PVN receive dense local GABAergic inputs from the bed nucleus of stria terminalis, preoptic area, and hypothalamus (Decavel and van den Pol 1992; Roland and Sawchenko 1993). Local synaptic inputs to PVN neurons are primarily GABAergic (Tasker and Dudek 1993). In the central neurocircuitry of stress, these GABAergic inputs are considered to relay the inhibitory information from forebrain limbic system nuclei such as the hippocampus, ventral subiculum, prefrontal cortex, and lateral septum to the PVN (Herman and Cullinan 1997). In the central regulation of sympathetic output, it has been suggested that the PVN GABAergic system serves as part of a negative feedback loop in the regulation of blood pressure (Ferguson and Latchford 2000) as it exerts a tonic inhibitory effect on sympathetic regulation of blood pressure (Martin et al. 1991), mediates nitric oxide (NO)–induced inhibitory effects on the renal sympathetic nerve activity (Zhang and Patel 1998) and displays reduced activity in the spontaneously hypertensive rats (Horn et al. 1998; Kunkler and Hwang 1995). In addition, the GABAergic synaptic activity in the
PVN is increased by NO (Bains and Ferguson 1997) and vasopressin (Hermes et al. 2000). Presently, it is not yet known whether NA can modulate the GABAergic inhibitory transmission in the PVN.

In this work, we examined the actions of NA on GABAergic synaptic currents recorded from type II PVN neurons of the rat brain slice and determined the type of adrenoceptors involved. The type II PVN neurons, considered to be putative parvocellular neurosecretory and preautonomic cells (Hoffman et al. 1991; Tasker and Dudek 1991), were identified on the basis of electrophysiological criteria (Luther and Tasker 2000; Tasker and Dudek 1991). Preliminary results of this work have been presented previously (Han et al. 1999; Ryu et al. 1998).

Methods

Slice preparation

Brain slices containing the PVN were prepared from male Sprague-Dawley rats (4–6 wk old) according to the methods reported previously (Tasker and Dudek 1991). Animal experiments were carried out according to the protocol for the care and use of animals approved by the Laboratory Animal Care Advisory Committee of Seoul National University. Rats were anesthetized by ether and quickly decapitated. The brain was dissected within 1 min and immersed in an oxygenated, ice-cold artificial cerebrospinal fluid (ACSF) for approximately 1 min. The composition of ACSF was (in mM) 126 NaCl, 26 NaHCO3, 5 KCl, 1.2 NaH2PO4, 2.4 CaCl2, 1.2 MgCl2, and 10 glucose. The hypothalamus was blocked with a razor, and one or two coronal hypothalamic slices (400 μm) were cut just caudal to the optic chiasm with a vibrating tissue slicer (WPI, Sarasota, FL). The slices were immediately transferred to a storage chamber and incubated for about 1 h. Then, one of the slices was transferred to a recording chamber (0.7 ml), where it was perfused (2 ml/min) with oxygenated (95% O2-5% CO2) ACSF.

Electrophysiological recording

Whole cell recording of neurons in the PVN was performed on the hypothalamic slices with or without visualization of individual neurons. Pipettes were pulled from borosilicate glass capillaries of 1.7 mm diam and 0.5 mm wall thickness. Open resistance ranged from 2 to 5 MΩ, and seal resistance ranged from 1 to 10 GΩ. Patch pipettes were filled with a solution containing (in mM) 140 KCl, 20 HEPES, 0.5 CaCl2, 5 EGTA, and 5 MgATP. The pH was adjusted to 7.2 with KOH (21 mM). For experiments determining the reversal potential of sIPSC, lidocaine N-ethyl bromide (QX-314, 5 mM) was added to the pipette solution to suppress the action potential firing in the recorded neurons (Fig. 2). For recording, a slice was placed in the recording chamber with a grid of nylon stocking threads supported by a U-shaped silver wire weight. Patch pipettes were positioned with the aid of a three-dimensional hydraulic micromanipulator (Narishige, Tokyo, Japan) into the presumed area of the paraventricular region of the PVN (Fig. 1C) under a dissection stereoscope (×10–40) for blind patch recording, or under an upright microscope with a differential interference contrast (BWS501W, Olympus, Tokyo, Japan) for visual patch recording. Among 121 neurons tested, 44 neurons were recorded by blind patch and 77 neurons by visual patch recording. Electrical signals were recorded with an Axoclamp 2B amplifier (probe gain, ×0.01 MU with HS-2 probe) or Axopatch 200B. For the resting membrane potential, the liquid junction potential (4.8 mV) was corrected according to Neher (1992). Current records were filtered at 1 kHz and digitized at 1 to ~5 kHz with an analog-digital converter (TL-1) and pClamp program (Version 6.03, Axon Instruments, Foster City, CA). Signals were also stored on videotape via a pulse code modulator (37 kHz, VR-10B, Instrutech, Port Washington, NY) for off-line analysis.

Cell identification

The neurons located in the medial one third of the PVN area between the third ventricle and the fornix, were targeted visually (Fig. 1C). Immediately after establishing the whole cell configuration, a series of 300-ms hyperpolarizing currents (−30 approximately −180 pA) was applied in current-clamp mode. In earlier experiments, neurons not showing low-threshold spikes (or rebound action potentials) after hyperpolarizing pulses larger than −100 mV for 300 ms were classified as type I, and the neurons responding with discrete or bursting low-threshold spikes were classified as type II according to Tasker and Dudek (1991). In later experiments, the types of neurons were determined by a series of depolarizing current pulses of 250 ms with a hyperpolarizing prepulse of 250 ms to approximately −100 mV (Luther and Tasker 2000). Neurons showing prominent transient outward rectification were classified as type I, and neurons showing little rectification as type II (Fig. 1, A and B). The patterns of noradrenergic modulation in the type II neurons, as classified by the earlier and later protocols, were identical. Therefore the results from two populations of type II neurons were pooled together in the analyses. Cells were excluded from analyses if they did not meet the
following criteria: input resistance near resting potential of ≥500 MΩ, resting membrane potential negative to −50 mV and spontaneous synaptic activity stable in frequency and amplitude.

**Recording and analysis of inhibitory postsynaptic currents (IPSCs)**

GABAergic IPSCs were recorded in the presence of nonselective glutamate receptor antagonists, kynurenic acid (1 mM), or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 20 μM) plus dl-2-amino-5-phosphonovaleric acid (AP5; 50 μM) at a holding potential of −70 mV. Alternatively, GABAergic postsynaptic currents were confirmed by a complete inhibition by bicuculline (20 μM), the GABA<sub>A</sub> receptor antagonist.

Measurement of the amplitude and frequency of IPSCs and the exponential fits of the decaying phases of synaptic currents were performed for a period of 180–300 s during the control and peak responses using Mini Analysis Program (Version 4.0, Synaptosoft Inc, Leonia, NJ). Distribution histograms of frequency, interevent intervals and amplitudes of spontaneous IPSCs (sIPSCs) were generated by the same program. A threshold for detection of sIPSCs was set at 20 pA for the amplitude and 300 pA·ms for the area of an IPSC. Under these conditions, more than 97% of the sIPSC events were counted with virtually no contamination by nonsynaptic currents. The individual IPSC peaks were counted first by a peak detection routine of the program with a Period-Start-Baseline of 6 ms, a time period before local maximum, to calculate average baseline. Any uncounted peaks were detected by eye under the manual mode. This procedure allowed detection of successive events separated by intervals as short as 4 ms. Peak amplitude was calculated by subtracting the average baseline from the amplitude at a local maximum. The decay time constant of a synaptic current was obtained by fitting the decaying phases of synaptic currents with a single exponential equation.

Drug-induced changes in the parameters of sIPSCs were normalized to the baseline values before the application of drugs (relative response). Experimental data are expressed as means ± SE, and the number of neurons tested and analyzed are represented by n. The statistical significance of data were determined using independent or paired Student’s t-test for the comparison of two means and the Kolmogorov-Smirnov two-sample test for distributions of the frequency, amplitude, and decay time constant. A level of P < 0.05 was considered to be significant.

**Drug application**

Drugs were added to the perfusing ACSF solution at known concentrations. When tested with blue ink solution, the solution was completely washed out in <2 min. Noradrenaline bitratear (1–1,000 μM), yohimbine hydrochloride (20 μM), prazosin hydrochloride (2–20 μM), phenylephrine hydrochloride (10–100 μM), clonidine (10–50 μM), bicuculline methiodide (20 μM), and kynurenic acid (1 mM) were purchased from Sigma (St. Louis, MO). CNQX (20 μM) and AP5 (50 μM) were obtained from Tocris Cookson (Bristol, UK), and tetrodotoxin (TTX, 1 μM) from Alomone Lab (Jerusalem, Israel). All drugs were dissolved directly in the ACSF except CNQX, which was dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO was not more than 0.05%.

**RESULTS**

A total of 121 type II PVN neurons with spontaneous IPSCs were tested by NA and/or other adrenergic agents in this work. The recorded cells had an average resting membrane potential of 58 ± 0.7 (SE) mV and input resistance of 757 ± 34 MΩ.

**Spontaneous GABAergic IPSC in type II PVN neurons**

The PVN neurons recorded demonstrated rich spontaneous synaptic activity at −70 mV. Figure 2 illustrates typical examples of spontaneous synaptic currents recorded with patch pipettes containing high Cl− (140 mM). The spontaneous postsynaptic currents were not affected by CNQX (20 μM), the antagonist of non−NMDA-type ionotropic glutamate receptors, but were blocked by bicuculline (20 μM), the antagonist of GABA<sub>A</sub> receptors (Fig. 2A). The lack of inflection in the cumulative probability curves shown in Fig. 2A suggest that there is only one population of synaptic events recorded. In a total of 22 neurons tested, CNQX (20 μM, n = 11) or kynurenic acid (0.04–1 μM, n = 11), the nonspecific antagonists of ionotropic glutamate receptors did not change the properties of the spontaneous postsynaptic currents. In addition, the spontaneous synaptic currents recorded in the presence of kynurenic acid were reversed at around 0 mV, which is close to the equilibrium potential for Cl− (0.6 mV, Fig. 2B). These results suggest that the major spontaneous synaptic currents in type II PVN neurons are GABA<sub>A</sub> receptor−mediated sIPSCs. The ranges of mean amplitude, frequency, and decay time constant of sIPSCs in individual type II neurons were 28–137 (76.9 ± 4.47) pA, 0.96–24.3 (4.9 ± 0.6) Hz, and 6.0–7.34 (11.2 ± 0.5) ms, respectively.

**Dual effects of NA on spontaneous GABAergic IPSCs**

**EXCITATORY EFFECTS ON S IPSC FREQUENCY.** In 71 of a total of 121 neurons tested, NA (100 μM) increased the frequency of sIPSCs to 356 ± 34% of the baseline value of 3.2 ± 0.5 Hz.

**FIG. 2.** Typical examples of spontaneous inhibitory postsynaptic currents (IPSCs) from type II PVN neurons. A: spontaneous inward synaptic currents recorded in a PVN neuron. Holding potential = −70 mV. Cumulative probabilities of amplitude and interevent intervals before and after application of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 20 μM) were not significantly different. Pipette solution contained 140 mM Cl−. B: spontaneous synaptic currents at various voltages. Na+−dependent action potentials were blocked by an internal QX-314 (5 mM).
The PVN neurons whose sIPSC frequency was enhanced by NA had a mean resting membrane potential and input resistance of $-57.6 \pm 0.9 \text{ mV}$ and $800 \pm 44 \text{ M}\Omega$, respectively. Figure 3A shows a typical current record showing an NA-induced increase in sIPSC frequency. Within 1 min after switching to the normal recording solution, sIPSCs were increased remarkably, and such effects lasted for 19.4 (8–37) min after switching to the normal recording solution. The sIPSCs plotted on an expanded time scale (Fig. 3B) further demonstrate the reversible increase of sIPSC frequency by NA. In addition, sIPSCs with larger amplitude were more frequently observed after application of NA in some neurons. The time course histogram of sIPSC frequency also indicated an immediate and reversible increase in sIPSC frequency (Fig. 3C), and the stimulatory effects of NA were repeatedly induced in a neuron (Fig. 3D, see also Fig. 7A). The effects of NA on sIPSC frequency (Fig. 3D) and its duration of action (Fig. 3E) were dependent on the concentrations of NA applied. The amplitude and decay time constant of sIPSCs were affected by NA in these neurons, but the changes were much smaller than those in the sIPSC frequency. The relative amplitudes of sIPSCs were increased by 16 $\pm 5.0\%$ (range, $-60$–$-132\%$, $P < 0.01$) from the baseline levels ($74 \pm 5.4 \text{ pA}$). Mean decay time constant was increased by 5 $\pm 2.0\%$ ($P < 0.01$) from the control value (11.9 $\pm 0.7 \text{ ms}$). However, the effects of NA on the amplitude and decay time constants showed little correlations with the effects of NA on the sIPSC frequency, so these were not analyzed further in this study.

**Inhibitory Effects on sIPSC Frequency.** In 40 of a total of 121 type II neurons tested, NA (100 $\mu\text{M}$) significantly decreased the sIPSC frequency to $46 \pm 4.3\%$ of the baseline value of $3.6 \pm 0.34 \text{ Hz}$ ($P < 0.001$). The mean resting potential and input resistance of these neurons were $-58 \pm 1.1 \text{ mV}$ and $792 \pm 47 \text{ M}\Omega$, respectively. Figure 4, A and B, illustrates the current records showing a typical inhibitory effect of NA on sIPSC frequency. The time course histogram of sIPSC frequency also indicated an immediate and reversible decrease in sIPSC frequency (Fig. 4C), and the inhibitory effects of NA could be repeatedly induced in a neuron (Fig. 4D, see also Fig. 7B). The recovery of sIPSC frequency occurred in 7.1 $\pm 0.6 \text{ min}$ of wash out with normal ACSF. The inhibitory effects of NA on sIPSC frequency (Fig. 4D) and its duration of action (Fig. 4E) were dependent on the concentrations of NA applied. In this group of neurons, the amplitudes of sIPSCs were not significantly changed by NA ($P = 0.63$), but decay time constant increased by 5 $\pm 1.9\%$ from their baseline levels (10.9 $\pm 0.60 \text{ ms}$, $P < 0.05$). There was no correlation between changes in frequency and decay time constant.

The holding currents of type II neurons were not changed by NA (100 $\mu\text{M}$) in 94% of type II neurons, but a reversible inward current (11 $\pm 2.4 \text{ pA}$) was observed in 7 of 121 neurons tested (data not illustrated). Among these neurons, the frequency of sIPSC was increased in four and decreased in three.

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**Fig. 3.** Noradrenergic increase of the frequency of sIPSCs. A: a typical current recording showing the effects of noradrenaline (NA, 100 $\mu\text{M}$) on sIPSCs in a type II neuron. Drugs were added at the time indicated by the horizontal bar. B: individual IPSCs from the same neuron shown in A before (a), during (b), and after (c) the application of NA are illustrated at an expanded time scale. C: time course of the frequency of sIPSCs shown in A for the whole recording period. The frequency was calculated every 30 s and plotted. D and E: concentration dependence of the effects of NA ($10^{-7}$–$10^{-3} \text{ M}$) on sIPSC frequency (D) or on the duration of stimulatory effects on sIPSC frequency (E) in 6 type II PVN neurons. The effects of NA on the sIPSC frequency were expressed as percentage of the frequency of the predrug control period at various NA concentrations. Duration of action of NA was determined as the period from the beginning to the recovery of the NA-induced increase in sIPSC frequency. Individual cells were marked by different symbols.

**$\alpha_1$-Adrenoceptor–mediated enhancement and $\alpha_2$-adrenoceptor–mediated reduction of sIPSC frequency**

To determine the type of adrenoceptors mediating the NA-induced modulation of sIPSC frequency, we further looked into the effects of NA in the presence of specific adrenoceptor antagonists. Figure 5 illustrates that the NA-induced enhancement of sIPSC frequency was blocked by prazosin, the $\alpha_1$-adrenoceptor antagonist (Fig. 5, A and C), whereas the NA-induced reduction of sIPSC frequency was blocked by yohimbine, the $\alpha_2$-adrenoceptor antagonist (Fig. 5, B and D). In the presence of prazosin (2 $\mu\text{M}$), the stimulatory effects of NA were blocked (Fig. 5, A and C). Figure 5E summarizes results from seven neurons, whose sIPSC frequency were increased to $235 \pm 26\%$ of the control ($1.9 \pm 0.2 \text{ Hz}$, $P < 0.05$). In addition, it is of note that, in three neurons treated with prazosin (2 at 2 $\mu\text{M}$ and 1 at 20 $\mu\text{M}$), the effect of NA was not only blocked but also reversed to decrease the sIPSC frequency as shown in Fig. 5A (marked by asterisk). Similarly, Fig. 5, B and D, illustrates that yohimbine (20 $\mu\text{M}$) blocked the
inhibitory effect of NA on sIPSC frequency. Figure 5F summarizes the blocking effects of yohimbine from six neurons in which sIPSC frequency was reduced to 38\% of the control (2.64 ± 0.19 Hz, \( P < 0.05 \)). We also observed a slight decrease in sIPSC frequency during application of yohimbine in three neurons, indicating an agonistic effect of yohimbine. Figure 6 further demonstrates that selective adrenoceptor agonists can produce the effects of NA on sIPSC frequency. Bath-application of phenylephrine, the \( \alpha_1 \)-adrenoceptor agonist (10–100 \( \mu \)M) or clonidine, the \( \alpha_2 \)-adrenoceptor agonist (10–50 \( \mu \)M) reversibly increased or decreased sIPSC frequency, respectively (Fig. 6, A–D). In 12 of 14 neurons tested at 10 (\( n = 3 \)) or 100 \( \mu \)M \( (n = 9) \), phenylephrine increased the frequency of sIPSC to 188 ± 27\% and 419 ± 37\% of the control \((P < 0.001, \) Fig. 6E, respectively. In 10 of 11 neurons tested at 50 \( \mu \)M, clonidine decreased sIPSC frequency to 43\% of the control \((P < 0.001, \) Fig. 6F), although clonidine at 10 \( \mu \)M did not significantly change sIPSC frequency \((n = 3) \). The above results collectively suggest that in type II PVN neurons \( \alpha_1 \)-adrenoceptors mediate the noradrenergic enhancement of sIPSC frequency, whereas \( \alpha_2 \)-adrenoceptors mediate the noradrenergic reduction in sIPSC frequency.

**Location of adrenoceptors on GABAergic presynaptic neurons**

To determine whether noradrenergic modulation of sIPSC frequency is dependent on action potential firing in the presynaptic GABAergic neurons, we tested NA in the presence of TTX (1 \( \mu \)M), the Na\(^+\) channel blocker that blocks neuronal firing. Figure 7, A, C, and E, illustrates that the stimulatory effects of NA on sIPSC frequency were blocked in the presence of TTX. The time course histogram shown in Fig. 7A shows that NA did not increase sIPSC frequency as in the control and wash out periods. Figure 7A also shows that the ongoing effect of the first NA was blocked by TTX (marked by

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**FIG. 4.** Noradrenergic decrease of sIPSC frequency. A: a typical current recording showing the inhibition of sIPSC frequency by NA (100 \( \mu \)M). B: detailed current records for IPSCs from the same neuron during control period and after application of NA as marked by \( a \rightarrow c \) in A. C: time course of the frequency of sIPSCs shown in A for the whole recording period. The frequency of sIPSC was measured every 10 s. Concentration dependence of the effects of NA (10\(^{-6}\)–10\(^{-4}\) M) on sIPSC frequency \((D) \) or on the duration of inhibitory effects on sIPSC frequency \((E) \) in 4 type II PVN neurons. Other descriptions are the same as in Fig. 3, D and E.

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**FIG. 5.** Effects of NA (100 \( \mu \)M) on the sIPSC frequency in the presence of adrenergic antagonists. A and B: time course histograms showing the blockade of the noradrenergic increase in sIPSC frequency by prazosin (2 \( \mu \)M, A), and that of the noradrenergic decrease by yohimbine (20 \( \mu \)M, B). C and D: current records of the same neurons before and after application of NA in the absence and presence of \( \alpha_1 \)-adrenoceptor antagonists, as indicated by arrows in A and B, respectively. E and F: mean blocking effects of prazosin \((E; n = 4 a t 2 \mu \text{M}, n = 3 a t 20 \mu \text{M}) \) and yohimbine \((F; n = 6 a t 20 \mu \text{M}) \) on respective NA-induced increase and decrease of sIPSC frequency, NA, noradrenaline; Pra 2 and Pra 20, 2 and 20 \( \mu \)M prazosin, respectively; yoh, yohimbine.
FIG. 6. Effects of α-adrenoceptor agonists on the frequency of sIPSCs. A and B: time course histograms of sIPSC frequency showing the stimulatory effects of phenylephrine (A) and the inhibitory effect of clonidine (B). The mean frequency was calculated every 30 and 10 s, respectively. C and D: current records before (a) and during (b) the effects of adrenergic agonists on sIPSC frequency as marked in A and B. E and F: mean effects of phenylephrine (E; n = 3 at 10 μM and n = 9 at 100 μM) and clonidine (D; n = 3 at 50 μM and n = 9 at 50 μM) on the frequency of sIPSCs, respectively. PE, phenylephrine; Clo, clonidine. * P < 0.05; ** P < 0.01; *** P < 0.001.

asterisk) 20 min after application of NA. Current records of 30 s from the same record (marked by arrows) illustrate that the NA-induced increase in sIPSC frequency is blocked in the presence of TTX (Fig. 7C). In the pooled results from four cells tested with NA (100 μM, n = 3) or phenylephrine (10 μM, n = 1), the sIPSC frequency was increased to 199 ± 28% of the control (2.20 ± 0.39 Hz, P < 0.01) in the absence of TTX, but surprisingly NA decreased the sIPSC frequency to 58 ± 12% of the control (P < 0.01) in the presence of TTX (Fig. 7E). In contrast, in five neurons whose sIPSC frequency was decreased by NA (100 μM), the inhibitory effects of NA were not blocked by TTX (Fig. 7, B and D). The mean of sIPSC frequency was significantly decreased to 38 ± 9 in the absence of TTX and 33 ± 6% of the control (5.30 ± 1.40 Hz) in the presence of TTX, respectively (Fig. 7F), and both the effects of NA on sIPSC frequency in the absence and presence of TTX were not significantly different. These results together indicate that the α1-adrenoceptor–mediated increase in sIPSC frequency is dependent on the action potential firing of presynaptic GABAergic neurons, but the α2-adrenoceptor–mediated decrease in sIPSC frequency is not.

Dual noradrenergic modulation in individual PVN neurons

Most neurons tested with NA showed a monophasic change in sIPSC frequency. However, some neurons showed a biphasic response to NA as illustrated in Fig. 8, A and C. The time course histogram of sIPSC frequency in a neuron tested with NA (100 μM) indicates an initial decrease to about 50%, and subsequent increase to about 300% of the predrug control response in the sIPSC frequency (Fig. 8B). Similar responses were observed in 11 neurons tested with NA (100 μM). The possibility of such dual noradrenergic modulation was also supported by the result shown in Figs. 5A and 7E, where the α1-adrenoceptor–mediated decrease in sIPSC frequency was unmasked in the presence of prazosin (2–20 μM) or TTX (0.1 μM) that blocked the α1-adrenoceptor–mediated response to NA. Furthermore, in 8 of 11 neurons tested, the sIPSC frequency of individual neurons was increased by the α1-adrenoceptor agonist, phenylephrine and decreased by the α2-adrenoceptor ag-
agonist, clonidine, respectively. When three of these eight neurons were tested with NA, one neuron showed \( \alpha_2 \)-adrenoceptor–mediated effect, while the rest of neurons displayed \( \alpha_1 \)-adrenoceptor–mediated effect. These results imply that the GABAergic inputs to a large subpopulation of type II PVN neurons are balanced by noradrenergic dual modulation which is mediated by activation of \( \alpha_1 \)- or \( \alpha_2 \)-adrenoceptors in the presynaptic GABAergic neurons.

**DISCUSSION**

We report here the effects of NA on GABAergic synaptic current, the major type of spontaneous synaptic activity in putative parvocellular PVN neurons. We found that NA induced two opposite effects on the frequency of sIPSC in the PVN; an \( \alpha_1 \)-adrenoceptor–mediated increase observed in 58% of the neurons and an \( \alpha_2 \)-adrenoceptor–mediated decrease in 33% of the neurons tested with NA. Evidence supporting the involvement of \( \alpha_1 \)-adrenoceptors includes the blockade of NA-induced increase of sIPSC frequency by the \( \alpha_1 \)-adrenoceptor antagonist and the increase of sIPSC frequency by the \( \alpha_1 \)-adrenoceptor agonist. Evidence for the involvement of \( \alpha_2 \)-adrenoceptors includes the blockade of NA-induced decrease of sIPSC frequency by \( \alpha_2 \)-adrenoceptor antagonist and the inhibition of sIPSC frequency by the \( \alpha_2 \)-adrenoceptor agonist. Our observation that TTX blocked only the stimulatory effects of NA on sIPSC frequency also suggests that \( \alpha_1 \)-adrenoceptors are present on the cell body, whereas \( \alpha_2 \)-receptors are present on the axon terminals of presynaptic GABAergic neurons in the PVN. In addition, we found that the noradrenergic dual modulation of sIPSC frequency commonly occurred in individual PVN type II neurons tested with the selective adrenergic agonists or NA in the presence of \( \alpha_1 \)-adrenoceptor antagonist or TTX.

**Noradrenergic dual modulation of GABAergic synaptic transmission**

In more than one-half of type II PVN neurons tested here, NA increased sIPSC frequency via \( \alpha_1 \)-adrenoceptors, but in one-third of the neurons NA decreased sIPSC frequency via \( \alpha_2 \)-adrenoceptors. The electrical properties of these neurons were not significantly different in their baseline frequencies (3.2 vs. 3.6 Hz), amplitudes (74.0 vs. 82.6 pA), and decay time constants of sIPSC (11.9 vs. 10.9 ms), resting membrane potentials (57.6 vs. 58.2 mV), and input resistances (800 vs. 792 MΩ). In addition, the observation that over 90% of individual neurons tested responded to either \( \alpha_1 \)- or \( \alpha_2 \)-receptors, and both \( \alpha \)-adrenoceptor agonists also indicates that such dual noradrenergic modulation of IPSC commonly occurs in most type II PVN neurons. When tested with NA, the mixed \( \alpha \)-adrenoceptor agonist, the sIPSC frequency in PVN type II neurons was either increased or decreased or first decreased and then increased. Therefore it is likely that the differences in noradrenergic response patterns in type II PVN neurons were determined by the balance of two \( \alpha \)-adrenoceptor subtypes in the presynaptic GABAergic neurons rather than by the intrinsic differences in the type II PVN neurons. The relative dominance of \( \alpha \)-adrenoceptor subtypes could be an intrinsic property of GABAergic inputs to the PVN from various origins (Roland and Sawchenko 1993), or could reflect the influences of hormones such as glucocorticoids that shifted the release pattern of CRH from \( \alpha_2 \)- to \( \alpha_1 \)-adrenoceptor–mediated responses in cultured hypothalamic slices (Feuvrier et al. 1998; Szafarczyk et al. 1995). Presently, it is not known what determines such synaptic response patterns to NA, or the expression of specific types of adrenoceptors in the presynaptic GABAergic cells.

Previously, it was shown that NA increases the frequency of spontaneous excitatory synaptic potential (sEPSP) in one-third of type II PVN neurons via \( \alpha_1 \)-adrenoceptors (Daftary et al. 2000). The present study further extends our understanding of the noradrenergic modulation of synaptic transmission in parvocellular neurons of the PVN by showing a dual modulation of NA on sIPSC frequency in type II PVN neurons. The noradrenergic modulation of sIPSC occurs widely in type II PVN neurons as NA-induced changes in sIPSC frequency were observed in over 90% of neurons tested. Since the activation of GABA\(\gamma\)-receptor channels hyperpolarizes neuronal membranes or effectively counteracts excitatory inputs of different origins, it is highly likely that the noradrenergic modulation of tonic GABAergic inputs plays major roles in regulation of the excitability of parvocellular neuroendocrine neurons in the PVN (Herman and Cullinan 1997). However, further study is needed to understand the specific physiological demands for the differential activation of \( \alpha_1 \)-adrenoceptors in the presynaptic glutamatergic or GABAergic neurons.

In other hypothalamic nuclei, it has been shown that NA decreases sIPSC frequency through the activation of presynaptic \( \alpha_2 \)-adrenoceptors in hypothalamic supraoptic nucleus (Wang et al. 1998) and NA, released by electrical stimulation of the A1 cell group, increases GABA outflow via \( \alpha \)-adrenoceptors in the medial preoptic area (Herbison et al. 1990). In other brain areas, the activation of \( \alpha \)- or \( \alpha_1 \)-adrenoceptors in
presynaptic neurons has been shown to increase sIPSC frequency in the sensory motor cortex (Bennett et al. 1998) and the frontal cortex (Kawaguchi and Shindou 1998). In hippocampal CA1 pyramidal cells, NA decreased the amplitude of evoked IPSPs via presynaptic α2-adrenoceptors, but increased sIPSP frequency (Madison and Nicoll 1988). It is also known that activation of β-adrenoceptors can increase sIPSC frequency in the cerebella stellate cells (Kondo and Marty 1998). On the other hand, the activation of α2-adrenoceptors in presynaptic terminals decreased IPSC frequency in neurons of the olfactory bulb (Trombley and Shepherd 1992), or EPSC frequency in the hippocampus (Boehm 1999). These reports collectively suggest that the GABA release process is a common target for noradrenergic modulation in the CNS.

**Functional significance of noradrenergic modulation of GABAergic inhibitory postsynaptic currents**

The presence of sIPSC in most type II PVN neurons studied in this work indicates that these neurons are under a tonic GABAergic inhibition because GABA_A receptor activation inhibits neuronal excitability in central neurons. Furthermore, the α1-adrenoceptor-mediated enhancement of sIPSC frequency would decrease and α2-adrenoceptor-mediated reduction of sIPSC frequency would increase the excitability of type II PVN neurons. Since the type II PVN neurons have been considered as parvocellular neurons (Hoffman et al. 1991; Tasker and Dudek 1991), the neurons studied in this work could include 1) neuroendocrine cells that could project to anterior pituitary and release hypothiostropic hormones or 2) the preautonomic cells that project to spinal cord and modulate visceral organs (Swanson and Kuyper 1980).

If the type II PVN neurons recorded here were neurosecretory cells releasing hypothiostropic hormones such as CRH, the hormone release would be inhibited by activation of α1-adrenoceptors, but enhanced by activation of α2-adrenoceptors on the presynaptic GABAergic neurons. In relation to the regulation of hypothalamus-pituitary-adrenal axis, our findings are in agreement to the earlier results indicating a noradrenergic inhibition of CRH (Tuomisto and Mannisto 1985). But, our results are not consistent with the later results indicating α1-adrenoceptor-mediated increase and α2-adrenoceptor-mediated decrease in CRH secretion (Plotsky et al. 1989). This discrepancy could be due to other factors that can affect CRH release from parvocellular PVN neurons such as glutamatergic inputs, direct actions of NA on adrenoceptors of PVN cell membrane (Daftary et al. 2000), and differences in experimental conditions (Plotsky et al. 1989; Whitnall 1993). In addition, it is likely that the GABAergic pathways studied here may account for parts of negative feedback inputs (Meister et al. 1988; Meister et al. 1988) from the recurrent collaterals of CRH neurons (Liposits et al. 1985; Silverman et al. 1989). However, further studies are needed to prove the negative feedback inputs to identified parvocellular neurosecretory cells.

Alternatively, if the type II PVN cells studied here were the preautonomic neurons, the α1- and α2-adrenoceptor-mediated changes in sIPSC frequency would decrease and increase the central sympathetic outflow to the medulla and spinal cord, respectively. The presence of active spontaneous GABAergic synaptic inputs seen in this study agrees well with the finding that the injection of bicuculline into the PVN enhanced cardiovascular activity and plasma catecholamines (Martin et al. 1991), suggesting that PVN preautonomic neurons are under GABAergic inhibition. It has been reported that GABA mediates inhibitory effects of NO on the renal sympathetic nerve activity (Zhang and Patel 1998), and that GABA binding sites (Kunkler and Hwang 1995) and glutamate decarboxylase levels (Horn et al. 1998) are lower in the hypothalamus of spontaneously hypertensive rats. In this case, it is likely that the GABAergic inhibitory pathway acts as a local target for NA inputs (Ebihara et al. 1993; Harland et al. 1989) in determining central sympathetic outflow (Ferguson and Latchford 2000).

Finally, we cannot exclude the possibility that the PVN interneurons (Daftary et al. 1998; van den Pol 1982) were included in our neurons. In this case, it will be more complicated to make any physiological interpretation from the noradrenergic modulation of sIPSC frequency since little information is available on the property of interneurons in the parvocellular division of the PVN. Therefore to further understand the functional significance of the catecholaminergic modulation of sIPSCs in the PVN, it will be necessary to determine the electrical and chemical phenotype, and the synaptic targets of type II PVN neurons.

Our data suggest that the majority of type II PVN neurons receive tonic inhibitory synaptic inputs from NA-sensitive GABAergic neurons, but it is not precisely known where they are localized. Presynaptic GABAergic neurons could originate from the GABAergic neurons inside the PVN and/or the proximal limbic areas to the PVN such as the dorsomedial, anterior hypothalamic and preoptic areas, and the bed nucleus of the stria terminalis, which are known to relay inhibitory information from the limbic system (Boudaba et al. 1996; Cullinan et al. 1993; Roland and Sawchenko 1993). In our coronal slice preparation, it is likely that presynaptic GABAergic neurons originated from the lateral hypothalamic area, the posterior nucleus of the stria terminalis (Boudaba et al. 1996), and the PVN itself. If the presynaptic GABAergic neurons are located inside the PVN, our findings may indicate the presence of a local feedback circuit in the PVN (Liposits et al. 1985; Silverman et al. 1989), or that of inhibitory inputs from proximal limbic areas to the PVN (Boudaba et al. 1996; Cullinan et al. 1993; Roland and Sawchenko 1993).

Neurons in the PVN have rich GABAergic synapses (Decavel and van den Pol 1990) and projections from the peri- and intranuclear regions of the PVN (Boudaba et al. 1996; Cullinan et al. 1993; Roland and Sawchenko 1993). Our findings strongly indicate that such GABAergic synaptic inputs to most PVN type II neurons are under a dual noradrenergic modulation.

We thank Dr. Allan E. Herbison for critical reading of the manuscript and Drs. Jun-Ho Nah and In-Koo Huang for technical assistance in morphological identification of PVN neurons. This work was supported by the Korea Ministry of Science and Technology under the Brain Science Research Program and in part by the Brain Korea 21 Project.

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