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Noradrenergic Modulation of Spontaneous Inhibitory Postsynaptic Currents in the Hypothalamic Paraventricular Nucleus

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Previous studies have suggested that brain stem noradrenergic inputs differentially modulate neurons in the paraventricular nucleus (PVN). Here, we compared the effects of norepinephrine (NE) on spontaneous GABAergic inhibitory postsynaptic currents (sIPSCs) in identified PVN neurons using slice patch technique. In 17 of 18 type I neurons, NE (30~100 μM) reversibly decreased sIPSC frequency to 41±7% of the baseline value (4.4±0.8 Hz, $p<0.001$). This effect was blocked by yohimbine (2~20 μM), an α_2-adrenoceptor antagonist and mimicked by clonidine (50 μM), an α_2-adrenoceptor agonist. In contrast, NE increased sIPSC frequency to 248±32% of the control (3.06±0.37 Hz, $p<0.001$) in 31 of 54 type II neurons, but decreased the frequency to 41±7% of the control (5.5±1.3 Hz) in the rest of type II neurons ($p<0.001$). In both types of PVN neurons, NE did not affect the mean amplitude and decay time constant of sIPSCs. In addition, membrane input resistance and amplitude of sIPSC of type I neurons were larger than those of type II neurons tested (1209 vs. 736 MΩ, $p<0.001$; 110 vs. 81 pS, $p<0.001$). The results suggest that noradrenergic modulation of inhibitory synaptic transmission in the PVN decreases the neuronal excitability in most type I neurons via α_2-adrenoceptor, however, either increases in about 60% or decreases in 40% of type II neurons.

Key Words: Paraventricular nucleus, Norepinephrine, IPSC, Alpha adrenoceptor, Clonidine, Yohimbine

INTRODUCTION

The paraventricular nucleus (PVN) of the hypothalamus is the major integrating site for the endocrine and autonomic function (Swanson & Sawchenko, 1980). Based on the common output connections, peptide expression, and/or location in the nucleus, the neurons in the PVN are generally grouped into magnocellular and parvocellular neurons and segregated into specific anatomical compartments (Swanson & Sawchewnko, 1983; Armstrong, 1995). Magnocellular neurons project to the posterior pituitary and release oxytocin or vasopressin. Parvocellular neurons can be either neuroendocrine or preautonomic cells. The neuroendocrine parvocellular cells that project to the external median eminence, regulate the release of hormones from the anterior pituitary by secreting various releasing or inhibiting hormones such as corticotropin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH), growth hormone-releasing hormone, somatostatin and dopamine. The preautonomic parvocellular neurons project to autonomic centers in the brain stem and spinal cords that are involved in the regulation of heart rate and blood pressure (Swanson & Sawchewnko, 1983; Armstrong, 1995). Some of these neurons are also peptidergic (Cechetto & Saper, 1988; Hallbeck et al, 2001).

Previous electrophysiological and morphological studies have shown that PVN neurons can be divided into two groups: type I and type II (Tasker & Dudek, 1991). Type I neurons express a transient outward rectification in the current clamp recording (Tasker & Dudek, 1991), caused by a transient A-type potassium current (Luther & Tasker, 2000). These neurons have been identified as putative magnocellular neurosecretory cells (Hoffman et al, 1991) that express oxytocin and vasopressin. Electrophysiologically, oxytocin neurons can be distinguished from vasopressin neurons of the PVN (Stern & Armstrong, 1995; Cui et al, 2000). Type II neurons do not express the transient outward rectification and some cells generate a variable low threshold spike caused by a T-type calcium current (Tasker & Dudek, 1991; Luther & Tasker, 2000). In the immunohistochemical study, type II neurons have been identified as putative parvocellular neurons (Hoffman et al, 1991). Recent electrophysiological studies further revealed the presence of subtypes of both type I and type II PVN neurons. For example, type I neurons are classified into two subgroups based on the degree of outward rectification (Luther et al, 2000). The presence of low threshold spike became a common property of the type II neurons projecting to the brainstem and spinal cord (Cui et al, 2001, Stern, 2001) that could be further classified into three distinct neuronal groups (Stern, 2001).

The differences in the intrinsic electrophysiological pro-

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ABBREVIATIONS: PVN, paraventricular nucleus; NE, norepinephrine; sIPSC, spontaneous inhibitory postsynaptic current; ACSF, artificial cerebrospinal fluid; GABA, γ-aminobutyric acid; EPSC, excitatory postsynaptic current; CRH, corticotropin releasing hormone.

perties of neuronal membranes between type I and type II PVN neurons will determine the excitability of PVN neurons, and the release of neurotransmitters and/or neurohormones and neuropeptides. Another important factor that affects the neuronal excitability is fast and slow synaptic currents mediated by various neurotransmitters released from the presynaptic neurons. PVN neurons receive both glutamatergic and GABAergic synaptic inputs. Such excitatory (Boudaba et al, 1997; Daftary et al, 1998; Cui et al, 2001) and inhibitory synaptic inputs (Tasker & Dudek, 1993; Boudaba et al, 1996; Cui et al, 2000; Hermes et al, 2000; Cui et al, 2001) to the identified PVN neurons have been also confirmed electrophysiologically.

Noradrenergic inputs from the brainstem noradrenergic cell groups (Sawchenko & Swanson, 1982; Cunningham & Sawchenko, 1988) are critical for the correct functioning of multiple hypothalamic neuronal networks. Norepinephrine (NE) signals maintain endocrine homeostasis including the networks for corticotropin releasing hormone (CRH) (Plotsky et al, 1989; Whitnall, 1993; Pacak et al, 1995), gonadotropin releasing hormone (Herbison, 1997) and oxytocin/vasopressin (Leng et al, 1999).

In PVN neurons, NE induces both stimulatory and inhibitory effects (Inenaga et al, 1986; Kim et al, 1989). In recent slice patch experiments, NE increased the frequency of excitatory postsynaptic currents in 42% of type I neurons and depolarized 23% of type I neurons. Both effects were mediated by α_1-adrenoceptors (Daftary et al, 1998). In type II neurons, NE was found to increase the frequency of excitatory synaptic potentials in 36% of neurons through α_1-adrenoceptors on local glutamatergic neurons, but hyperpolarized 14% of neurons via β-adrenoceptors on the cell body (Daftary et al, 2000). These results suggest that type I and type II PVN neurons may be under the different or opposite noradrenergic modulations. For spontantaneous inhibitory postsynaptic currents (sIPSCs), we have shown that NE increased the frequency of sIPSCs in 59% of type II PVN neurons via somatic or dendritic α_1-adrenoceptors, but decreased the frequency in 33% of type II neurons via axonal terminal α_2-adrenoceptors on the presynaptic GABAergic neurons in the PVN (Han et al, 2002). However, it is not known whether NE also affects sIPSCs of type I neurons, and, if so, whether the noradrenergic modulations of sIPSCs in type I neurons are different from those in type II neurons. The current study was conducted to differentiate the effects of NE on sIPSCs in type I and type II PVN neurons. A preliminary account of this data has been communicated previously (Chong et al, 2001).

METHODS

Slice preparation and maintenance

Brain slices containing the PVN were prepared from male Sprague-Dawley rats (4~6 weeks old) according to the methods reported previously (Tasker & Dudek, 1991). Animal experiments were carried out according to a 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 about 1 min. The composition of ACSF was (in mM): 126 NaCl, 26 $NaHCO_3$, 5 KCl, 1.2 NaH_2PO_4, 2.4 $CaCl_2$, 1.2 $MgCl_2$ and 10 glucose. The hypothalamus was blocked with a razor, and one or two coronal hypothalamic slices (250 μm) were cut just caudal to the optic chiasm with a vibrating tissue slicer (WPI, Sarasota, FL, USA). The slices were immediately transferred to a storage chamber and incubated for about 1 hour. Then, one of the slices was transferred to a recording chamber (0.7 ml), where it was perfused (2 ml/min) with oxygenated (95% O_2 − 5% CO_2) ACSF.

Electrophysiological recording

Whole-cell recording of neurons in the PVN was performed on the hypothalamic slices without or with visualization of individual neurons. Pipettes were pulled from borosilicate glass capillaries of 1.7 mm diameter and 0.5 mm wall thickness. Open resistance ranged from 2~5 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 $CaCl_2$, 5 EGTA and 5 MgATP. The solution pH was adjusted to 7.2 with KOH (21 mM). For the experiments to determine 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. 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 was positioned with an aid of a three-dimensional hydraulic micromanipulator (Narishige Co, Tokyo, Japan) into the presumed area of the parvocellular region of the PVN under a dissection stereoscope (10~40 X) for blind patch recording (Blanton et al, 1989), or under an upright microscope with a differential interference contrast (BW50WI, Olympus, Tokyo, Japan) for 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 10 kHz with an analog-digital converter (Digidata 1200A) and pClamp program (Version 8.0, Axon Instruments, Foster City, CA, USA).

Cell identification

The neurons located in the medial one third of the PVN area between the third ventricle and the fornix were targeted under the visual aid (Han et al, 2002). Immediately after establishing the whole-cell configuration, the type of neurons was determined by a series of depolarizing current pulses of 250 ms with a hyperpolarizing pre-pulse of 250 ms to around −100 mV, as described previously (Luther & Tasker, 2000). Type I neurons were differentiated from type II neurons based on the expression of a transient outward rectification (Fig. 1). Cells were excluded from analyses if they did not meet the following criteria: input resistance near resting potential of at least 500 MΩ, resting membrane potential negative to −50 mV and spontaneous synaptic activity stable in frequency and amplitude during pre-drug control period.

Membrane input resistance was calculated by dividing the voltage shift induced by hyperpolarizing current pulse applied to classify cell type. The amplitude of action potential was measured from the base line to peak level and the duration of action potential was measured at half ampli-

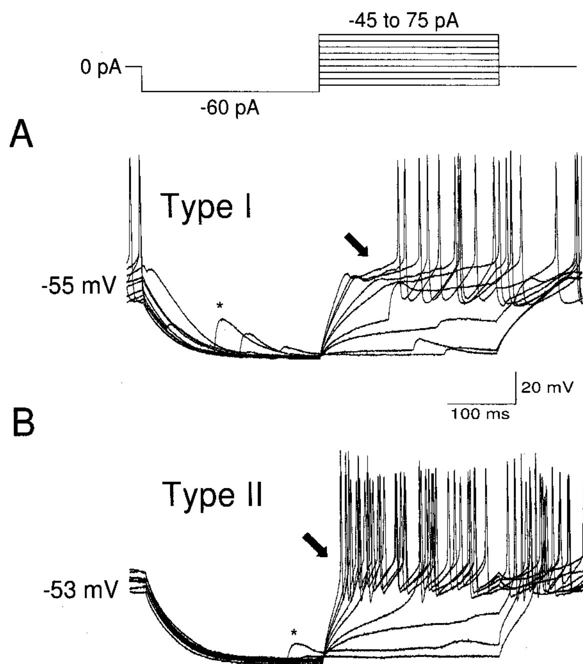

Fig. 1. Electrophysiological classification of type I and type II PVN neurons by a series of depolarizing current steps with a pre-pulse. A. A representative type I neuron responds to a series of incremental depolarizing current pulses (top) delivered at a hyperpolarized membrane potential with the expression of transient outward rectification, characterized by a dampening of the membrane charging curve and a delay to the 1st action potential (marked by an arrow). B. Type II neuron lacks the transient outward rectification (see arrow). The type II neuron displays electrotonic charging of its membrane in response to the same protocol. The asterisks indicate the reversed sIPSPs due to high internal Cl^- concentration.

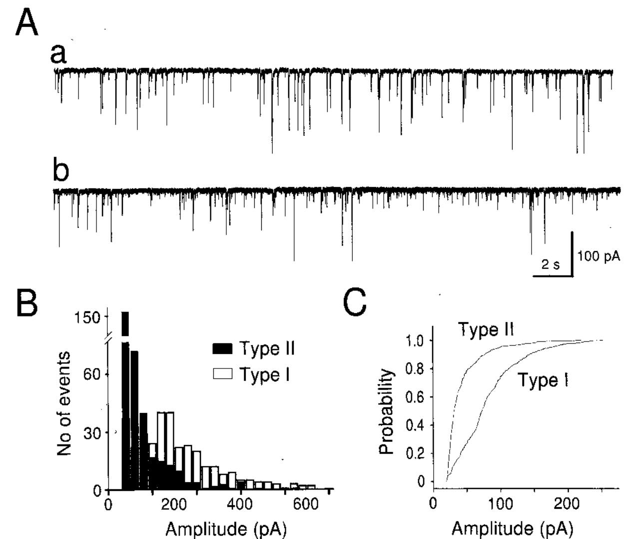

Fig. 2. Amplitudes of spontaneous IPSCs in the type I and type II PVN neurons. A. Current records showing representative sIPSCs from type I (a) and type II (b) neurons. B. Amplitude histograms of the sIPSCs in type I and type II neurons shown in A. C. Cummulative probability curves of sIPSC amplitudes in type I and type II. Note the shift of amplitudes of type I neurons (B and C) to the right.

tude level. Decay time constant of IPSCs were determined as the time to decay to $1/e$ of the peak.

Inhibitory postsynaptic currents

GABAergic inhibitory postsynaptic currents were recorded in the presence of nonselective glutamate receptor antagonists, CNQX (20 μM) plus 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 $GABA_A$ 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 120~180 s during the control and peak responses using Mini Analysis Program (Version 5.0, Synaptosoft Inc, Leonia, NJ, USA). Distribution histograms of frequency, inter-event intervals and amplitudes of sIPSCs were generated by the same program as described previously (Lee et al, 2001; Han et al, 2002). The neurons whose sIPSC amplitudes decreased by more than 20% were not included for analysis. Drug-induced changes in the parameters of sIPSCs were normalized to the baseline values before the application of drugs.

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 less than 2 min. Noradrenaline bitartrate (30~100 μM) was purchased from Sigma (St. Louis, MO, USA). Six-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 μM)) and DL-2-amino-5-phosphonovaleric acid (AP5, 50 μM) were obtained from Tocris Cookson (Bristol, UK). All drugs were dissolved directly in the ACSF.

Statistical analysis

Values are expressed as means ± SEM (standard errors of the mean) and the number of neurons tested and analyzed was represented by 'n'. Comparisons were made using the Student's unpaired t-test. The Student's paired t-test was used for two data sets from one recorded neuron. A level of $p<0.05$ was considered to be significant.

RESULTS

Electrophysiological characteristics of type I and type II PVN neurons

The results in this work are based on the data from a total of 18 type I neurons and 54 type II neurons that were successfully recorded and tested with NE. Among the electrophysiological properties determined, amplitude of sIPSCs of type I neurons were significantly larger than those of type II neurons as illustrated in Fig. 2. The representative records of sIPSCs in type I and type II neurons demonstrate that the sIPSCs of larger amplitudes

were more frequent in the type I neuron than in the type II neuron (Fig. 2A). The amplitude histogram (Fig. 2B) and cumulative probability curve of type I neurons shifted to the right compared to those of type II neurons. The mean amplitude of sIPSCs in type I and type II neurons were 110±8.8 pA and 81±6.2 pA, respectively ($p<0.001$). Similarly, the input resistance and amplitude of action potential of type I neurons were larger than those of type II neurons (1209±109 vs. 736±31 MΩ, $p<0.001$; 64±2.4 vs. 57±1.4 mV, $p<0.05$). However, resting membrane potential, frequency of action potential, and frequency and decay time constant of sIPSCs were not significantly different (Table 1).

Table 1. Electrophysiological properties of type I and type II PVN neurons (mean±SEM)

Parameters	Type I (n=17)	Type II (n=54)
RMP (mV)	−56.19±0.97	−58.09±0.69
Rm (MΩ)	1,208.62±109.05	736.31±30.77***
AP amplitude (mV)	64.23±2.44	57.18±1.36*
AP frequency (Hz)	19.0±1.27	22.9±1.32
AP duration (ms)	1.47±0.06	1.48±0.06
IPSC frequency (Hz)	4.43±0.83	4.12±0.60
IPSC decay time (ms)	10.68±0.74	10.93±0.46
IPSC amplitude (pA)	110.11±8.79	81.08±6.17***

RMP, resting membrane potential; Rm, input resistance; AP, action potential; IPSC, inhibitory postsynaptic current; *, $p<0.05$; ***, $p<0.001$.

NE has dual effects on the frequency of spontaneous IPSCs in type II neurons

Excitatory effect on sIPSC frequency: Fig. 3A shows a typical current record showing an NE-induced increase in sIPSC frequency. Within one minute after a bath application of NE, the frequency of sIPSCs increased remarkably and the effect of NE lasted for several minutes (Fig. 3B). Fig. 3C illustrates the actual records of sIPSCs during and after application of NE (100 μM), showing the increased number of events during application of NE. In 31 neurons out of total 54 type II neurons tested, NE (30~100 μM) significantly increased the frequency of sIPSCs to 248±32% of the baseline value of 3.06±0.37 Hz (Fig 3D, $p<0.001$). However, the amplitude and decay time constant of sIPSCs were not significantly changed by bath application of NE. The type II PVN neurons whose sIPSC frequency was enhanced by NE had mean resting membrane potential and input resistance of −57.8±0.9 mV and 704±42 MΩ, respectively.

Inhibitory effect on sIPSC frequency: In another group of type II PVN neurons, NE decreased the frequency of sIPSCs. Figs. 4A~4C illustrate typical current records and its time course histogram of sIPSC frequency obtained from a type II neuron whose sIPSC frequency was decreased by NE (100 μM). In 23 out of a total of 54 type II neurons tested, NE (30~100 μM) reversibly decreased the sIPSC frequency to 41±7% of the baseline value of 5.5±1.3 Hz

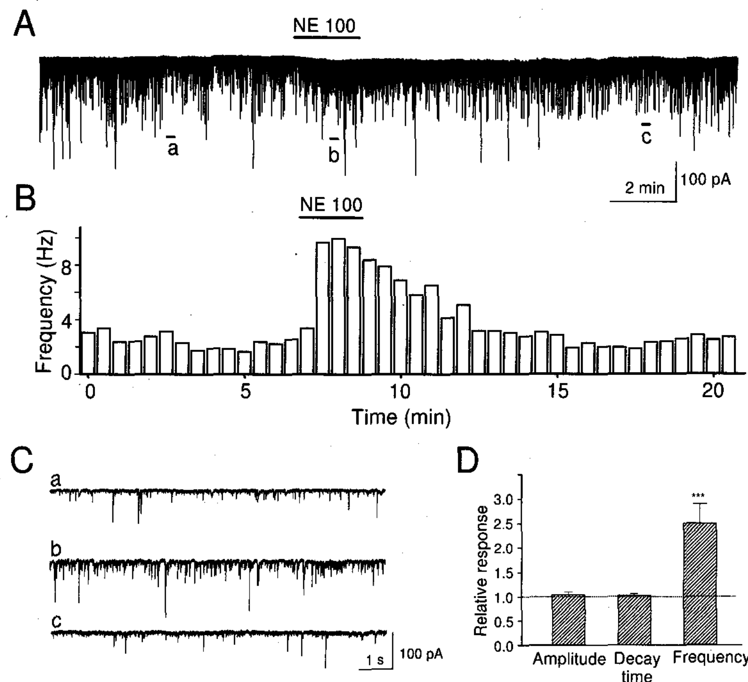

Fig. 3. Noradrenergic increase of the frequency of sIPSCs in type II neurons. A. A typical current record showing the effects of norepinephrine (NE, 100 μM) on sIPSCs in a type II neuron. Drugs were added at the time indicated by the horizontal bar. B. Time course of the frequency of sIPSCs shown in A for the whole recording period. The frequency was calculated every 30 s and plotted. C. Individual IPSCs from the same neuron shown in A before (a), during (b) and after (c) the application of NE illustrated at an expanded time scale. D. The effects of NE (100 μM) on sIPSC frequency, decay time constant and amplitude of sIPSCs (n=31, ***, $p<0.001$).

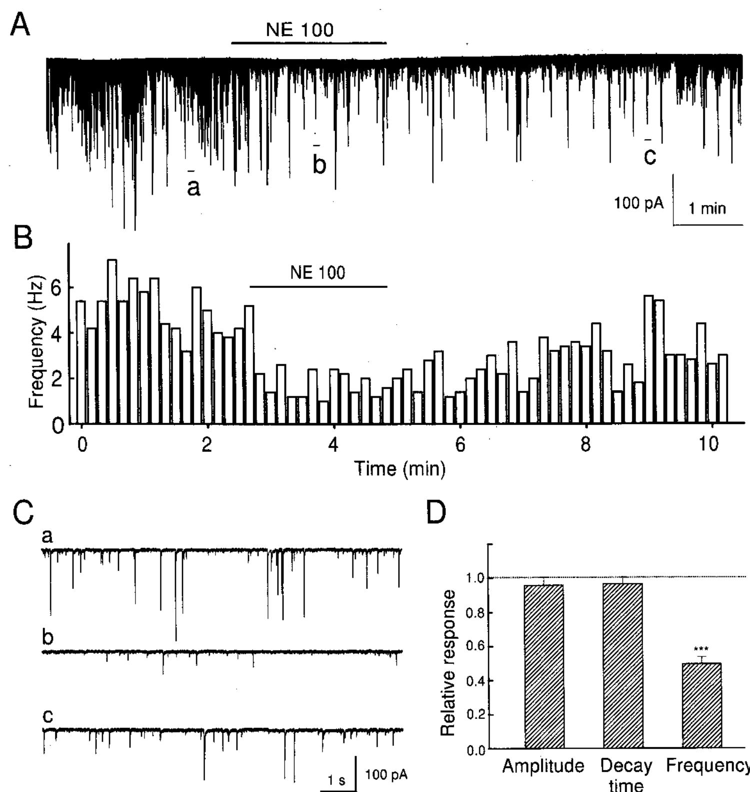

Fig. 4. Noradrenergic decrease of the frequency of sIPSCs in type II neurons. A. A typical current record showing the inhibition of sIPSC frequency by NE (100 μM). B. Time course of the frequency of sIPSCs shown in A for the whole recording periods. The frequency of sIPSC was measured every 30 s. C. Detailed current records for IPSC from the same neuron during control period and after application of NE, as marked by a~c in A. D. The effects of NE (100 μM) on sIPSC frequency, decay time constant and amplitude of sIPSCs (n=23, ***, $p<0.001$).

(Fig. 4D, $p<0.001$). However, NE did not change decay time constant and amplitude of sIPSCs. The mean resting potential and input resistance of these neurons were -58.4 ± 1.1 mV and 778 ± 44 MΩ, respectively. Mean values of resting membrane potential, input resistance, amplitude, decay time constant and frequency of sIPSCs were not different between two groups of type II neurons whose sIPSC frequency was increased and decreased by NE.

NE decreased the frequency of spontaneous IPSCs in type I PVN neurons

In contrast to type II neurons, NE induced only an inhibitory effect on the frequency of sIPSCs in type I PVN neurons. Figs. 5A~5C illustrate typical example of the current records for sIPSC in a type I neuron and its time course histogram, showing the NE-induced decrease in the frequency of sIPSCs. In 17 out of a total of 18 neurons tested, NE (30~100 μM) decreased the sIPSC frequency to 41 ± 7% of the baseline value of 4.4 ± 0.8 Hz (Fig. 5D, $p<0.001$). However, there were no remarkable changes in the amplitude and decay time constant of sIPSCs. The mean resting potential and input resistance of these neurons were -56.2 ± 1.0 mV and 1208 ± 109 MΩ, respectively.

Taken together, NE decreased the frequency of sIPSC in most type I neurons (94%). However, NE increased the frequency of sIPSC in 57% of type II neurons and decreased in the rest of type II neurons (Fig. 6).

NE-induced decrease in sIPSC frequency of type I neurons is mediated by α_2-adrenoceptors

Our results so far indicate that NE induces only inhibitory effects on sIPSC frequency in type I neurons, but induces both inhibitory and stimulatory effects in type II PVN neurons. As for the adrenoceptors mediating these dual effects, we previously showed the stimulatory and inhibitory effects of NE on sIPSC frequency in type II PVN neurons were mediated by α_1- and α_2-adrenoceptors, respectively (Han et al, 2002). However, the receptor mechanism of NE-induced decrease in sIPSC frequency in type I neurons has not not been known. Fig. 7 illustrates that the NE-induced decrease in sIPSC frequency in type I neuron was blocked by yohimbine (2~20 μM), an α_2-adrenoceptor antagonist (Fig. 7A and C). Furthermore, such inhibitory effect of NE was mimicked by clonindine (50 μM, an α_2-adrenoceptor agonist (Fig. 7B and D). However, the effects of clonidine were different from those of NE in that the inhibitory effects of clonidine lasted much longer (>20 min) in most cells tested. Fig. 7E and 7F summarize both the blocking effect of yohimbine (2~20 μM) from 5 type I neurons whose sIPSC frequency was reduced to 26 ± 4% of

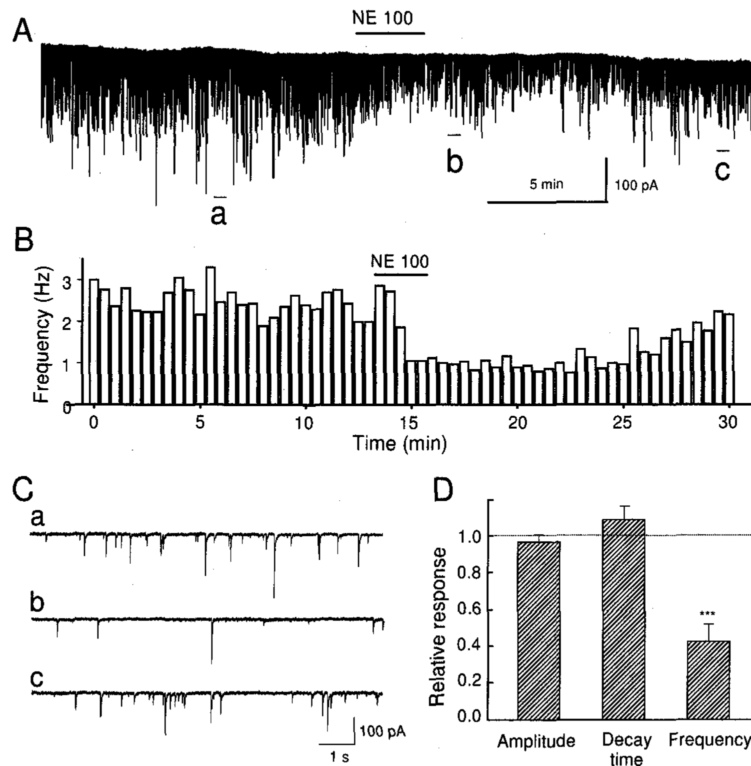

Fig. 5. Noradrenergic decrease of the frequency of sIPSCs in type I neurons. A. A typical current record showing the inhibition of sIPSC frequency by NE (100 μ M). B. Time course of the frequency of sIPSCs shown in A for the whole recording periods. The frequency of sIPSC was measured every 30 s. C. Detailed current records for IPSC from the same neuron during control period and after application of NE as marked by a~c in A. D. The effects of NE (100 μM) on sIPSC frequency, decay time constant and amplitude of sIPSCs (n=17, ***, $p<0.001$).

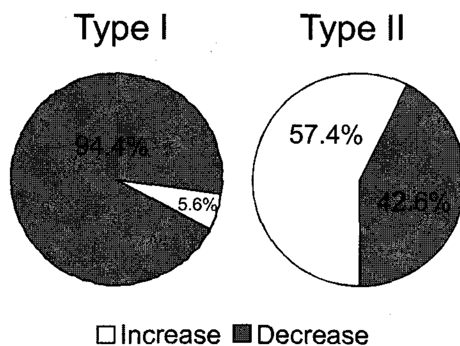

Fig. 6. Noradrenergic response patterns of the PVN neurons. The total number of type I and type II neurons tested were 17 and 54, respectively.

the control (4.60±1.39 Hz, $p<0.001$), and the inhibitory effect of clonidine (50 μM) from 3 type I neurons of which the sIPSC frequency was decreased to 58±7% of the control (6.11±1.68 Hz; $p<0.05$). The mean amplitudes and decay time constants of sIPSCs were not significantly changed by NE, yohimbine and clonidine. These results collectively indicate that the NE-induced decrease in sIPSC frequency in type I neurons is mediated by α_2-adrenoceptors.

DISCUSSION

The results showed that type I and type II PVN neurons were different in that membrane input resistance and amplitude of spontaneous IPSCs of type I PVN neurons were larger than those of type II neurons, and that the frequency of sIPSCs of most type I neurons was decreased, but that of type II neurons was either increased or decreased by NE. These findings further extend the previous criteria for the classification of the types of PVN neurons.

Differences in noradrenergic modulation of sIPSC frequency in type I and type II PVN neurons

In type II neurons, it has been shown that NE increased the frequency of sIPSCs via α_1-adrenoceptors of presynaptic GABAergic neurons in 59% of neurons tested, but decreased it via α_2-adrenoceptors of GABAergic neurons in 30% of neurons (Han et al, 2002). Such trend was also confirmed in this study: NE-induced increase in 57% and decrease in 43% of sIPSC frequency of type II PVN neurons. In type I neurons, our results revealed for the first time that NE decreased the frequency of sIPSCs in over 90% of neurons tested and that the inhibitory effect was mediated by α_2-adrenoceptors of GABAergic neurons. This finding is consistent with the reduction of sIPSC frequency in most magnocellular neurons of the supraoptic nucleus (Wang et al, 1998) that are similar to type I PVN neurons

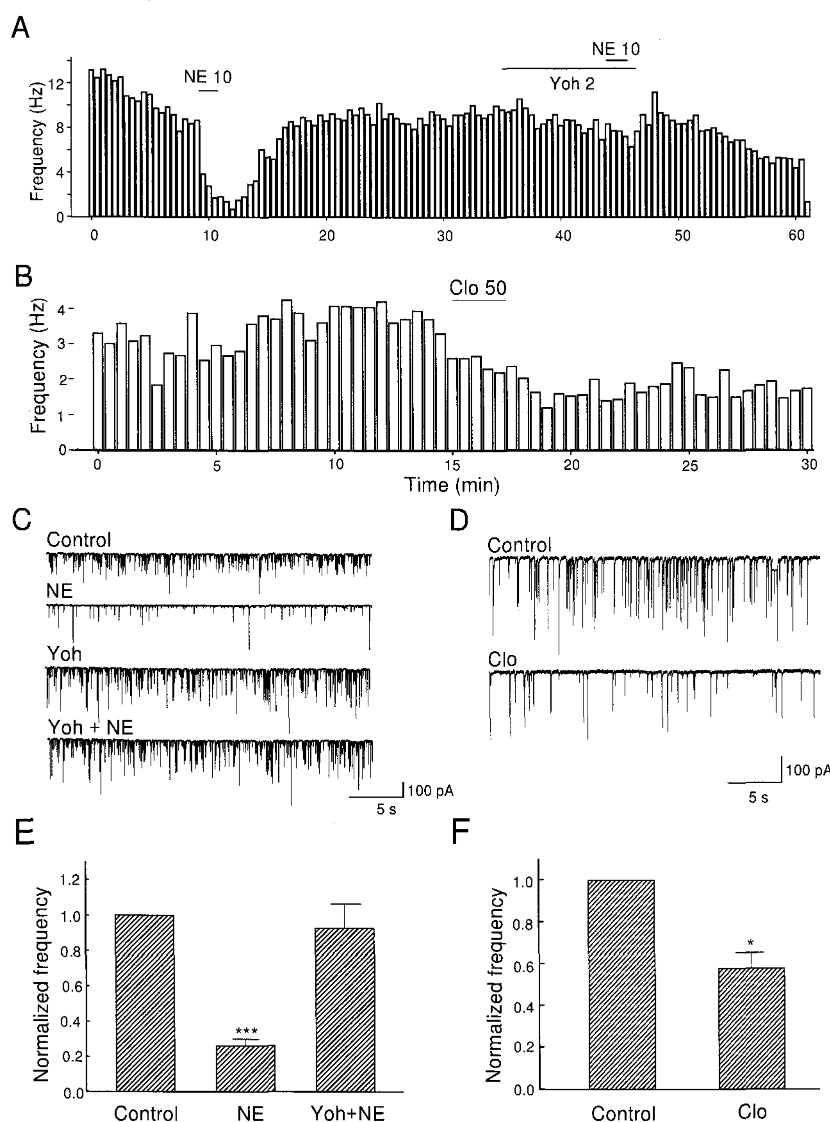

Fig. 7. Effects of α_2-adrenoceptor antagonist and agonist on sIPSC frequency in type I neurons. A and B: Time course histograms showing the blockade of the noradrenergic decrease in sIPSC frequency by yohimbine (2 μM) in a type I neuron whose sIPSC frequency was reduced by NE (10 μM, A), and the inhibition of sIPSC frequency by clonidine (50 μM) in another type I neuron (B). C. Current records from the neuron in A before and after application of NE in the absence and presence of yohimbine(C). D. Current records from the neuron in B before and after application of clonidine (D). E and F. Mean blocking effects of yohimbine (2 μM) on NE-induced decrease in sIPSC frequency in 5 type I neurons (E) and average effects of clonidine (50 μM) on sIPSC frequency in 3 type I neurons (F). NE, norepinephrine; yoh, yohimbine; clo, clonidine. *, $p<0.05$; ***, $p<0.001$.

in their function and morphology (Armstrong, 1995). Because the GABAergic IPSCs were recorded in the presence of antagonists of ionotropic glutamate receptors, the modulation of GABAergic synaptic currents by noradrenaline (Han et al, 2002) or vasopressin (Hermes et al, 2000) were likely to be monosynaptic events. In case of EPSC, NE increased the frequency of excitatory postsynaptic current via α_1-adrenoceptors in 42% of type I (Daftary et al, 1998) and 36% of type II neurons (Daftary et al, 2000). Currently, it is not clear whether these stimulatory effects of NE on the frequency of EPSC were induced mono-synaptically or poly-synaptically because these response could not be observed in the presence of TTX or blockers of ionotropic glutamate receptors (Daftary et al, 1998, 2000). Further study is also needed to elucidate anatomical and functional differences between the type II neurons whose sIPSC frequency was increased and those whose sIPSC frequency was decreased by noradrenaline.

Functional significance of differential noradrenergic modulation of sIPSC frequency in PVN neurons

The results of this study and Han et al (2002) showed

that most GABAergic inputs (>90%) to both types of PVN neurons were under the noradrenergic modulations, suggesting that GABAergic transmission was the major target of noradrenergic modulation in the PVN. Since we observed spontaneous IPSCs (~4 Hz) in most type I and type II neurons, it appears that both type I and type II PVN neurons are under a steady GABAergic inhibition in resting state.

In type I neurons, the noradrenergic inhibition of GABAergic IPSCs as described in this work and noradrenergic stimulation of excitatory synaptic transmission (Daftary et al, 1998) would increase the neuronal excitability in most neurons. Since type I neurons were identified as putative magnocellular neurons expressing oxytocin or vasopressin (Hoffman et al, 1991; Daftary et al, 1998), one can expect that the inhibition of sIPSC frequency by NE can cause an increase in the release of oxytocin or vasopressin at the posterior hypophysis. This observation is in good agreement with the previous reports indicating the noradrenergic increase in the release of oxytocin via α_1-adrenoceptors (for review see Leng et al, 1999). In the PVN, our results suggest, for the first time, that α_2-adrenoceptors also can mediate the NE-induced increase in the release of oxytocin and/or vasopressin by decreasing the frequency of sIPSCs in the type I neurons. Further study is needed to understand the relative contributions of α_1- and α_2-adrenoceptors to the neurosecretory function of type I neurons in the PVN.

In type II neurons, our results indicate that the noradrenergic modulation of sIPSC frequency can result in the increase in the neuronal excitability in some neurons, but result in the decrease in other group of neurons. Although type II PVN neurons are considered as putative parvocellular neurons (Hoffman et al, 1991), only limited information is available on the electrical properties, their projection and peptide expression (Cui et al, 2001; Stern, 2001). If the type II PVN neurons recorded here were neurosecretory cells releasing hypophysiotropic hormones such as CRH, the hormone release would be inhibited by activation of α_1-adrenoceptors, but enhanced by activation of α_2-adrenoceptors on the presynaptic GABAergic neurons. In relation to the regulation of hypothalamus-pituitary-adrenocortical axis, however, our findings appear to be opposite to the well-established effect of NE on the release of CRH: α_1-adrenoceptor activation increases and α_2-adrenoceptor activation decreases the secretion of CRH (Plotsky et al, 1989; Whitnall, 1993). Therefore, it is likely that the noradrenergic increase in the release of CRH is due to the stimulation of excitatory inputs to type II PVN neuron rather than the decrease in the GABAergic inhibitory inputs. Alternatively, if the type II PVN cells studied here were the preautonomic neurons, the α_1- and α_2-adrenoceptor-mediated changes in sIPSC frequency would decrease and increase the central sympathetic outflow to the 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 neurons are under a tonic inhibition by GABAergic system. It has been reported that GABA mediates inhibitory effects of NO on the renal sympathetic nerve activity (Zhang & Patel, 1998), and that GABA binding sites (Kunkler & 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 NE inputs (Harland et al, 1989; Ebihara et al, 1993) in determining central sympathetic outflow (Ferguson & Latchford, 2000).

Recently the preautonomic PVN neurons previously classified as type II were selectively identified in brain slice by using retrograde fluorescent dyes, and its morphology and electrophysiological properties were studied in detail (Cui et al, 2001; Stern, 2001). For more decisive understanding of noradrenergic modulation of specific groups of parvocellular neurosecretory PVN neurons, it would be necessary to identify specific neuron groups of known projection and chemical phenotype in routine electrophysiological experiments by using fluorescence reporter such as green fluorescent protein expressed in the CRH neurons (Spergel et al, 2001).

Differences in sIPSC amplitude and input resistance of type I and type II PVN neurons

The results showed that the amplitude of spontaneous IPSCs in type I neurons was larger than that in type II neurons (110 vs. 81 pA). To the best of our knowledge, this is the first to report the difference in sIPSC amplitude between type I and type II PVN neurons. Since GABAergic current of larger amplitude can induce stronger inhibition of the excitability of neurons, it is likely that basal electrical excitability of type I neurons is more tightly suppressed. In magnocellular neurons, the role of such GABA effect may be to favor the expression of the stereotyped suckling-induced bursting activity by attenuating inputs unrelated to suckling which are incompatible with bursts (Moos, 1995).

In general, the mechanisms to account for the variability in miniature IPSC amplitudes include the possibility of multi-quantal transmitter release, variation in transmitter content between vesicles, the stochastic behavior of channel gating, or differences in postsynaptic receptor number at different sites (Nusser et al, 1997; Cherubini & Conti, 2001). The sIPSCs, if not all, can represent miniature IPSCs because the frequency of sIPSCs was not changed in most neurons tested under the similar experimental conditions (Han et al, 2002). Therefore, it will be interesting to study whether there is any difference in these mechanisms for the variability of amplitudes between type I and type II cells.

Our results also indicate that the input resistance of type I neurons is larger than that of type II neurons (1209 vs. 736 MΩ). It is not yet known whether the lower sIPSC amplitude is due to lower input resistances of type II neurons, as described before. The reported input resistances of type I neurons measured with patch electrode are 772 MΩ (Cui et al, 2001) and 871 MΩ (Daftary et al, 1998), 789 MΩ (Li & Ferguson, 1996), 864 MΩ (Cui et al, 2000), 1027~1130 MΩ (Hermes et al, 2000), 1057 MΩ (Luther et al, 2000) and 1203 MΩ (Luther & Tasker, 2000). Those of type II neurons are 808 MΩ (Daftary et al, 2000) and 1,227 MΩ (Luther & Tasker, 2000). The input resistance of type II neurons projecting to spinal cord or dorsal medulla was reported to be 509 MΩ (Cui et al, 2001) and those of type II neurons projecting to the dorsal medulla were 626, 674 and 1,016 MΩ in group A, B and C neurons (Stern, 2001). These values indicate that input resistance is highly variable depending on the laboratories and even the studies from the same laboratory. Our results are

consistent with those of Cui et al (2001; type I vs. type II= 864 vs. 509 MΩ), but not with the results of Luther & Tasker (2000; 1203 vs. 1227 MΩ). This discrepancy may be due to heterogenity of type II neurons, as demonstrated in the work of Stern (2001). In motor neurons, the excitability of neurons is reduced, when membrane input resistance decreases during postnatal development, which is due to advent of synaptic inputs (Nunez-Abades et al, 2000) such as GABA and glycine or various ionic conductances (Viana et al, 1994; Berger et al, 1996). In the PVN, neurons receive dense local GABAergic inputs from the bed nucleus of stria terminalis, preoptic area and hypothalamus (Decavel & van den Pol, 1992; Roland & Sawchenko, 1993). But, little attention has so far been given to the differences in input resistances and contribution of GABAergic transmission to the excitability of type I and type II PVN neurons.

In summary, type I and type II PVN neurons were different in their noradrenergic modulation pattern and intrinsic property such as input resistance and the amplitude of sIPSCs. In type I PVN neurons, NE decreased sIPSC frequency via α_2 adrenoceptors in over 90% of neurons tested. In type II PVN neurons, NE increased sIPSC frequency in about 60% of neurons, but decreased the frequency in about 40% of neurons tested. These results indicate that the type I and II PVN neurons could be further differentiated by their local inhibitory synaptic input and its noradrenergic modulation.

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