Potentiation of glutamatergic synaptic input to supraoptic neurons by presynaptic nicotinic receptors

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Li, De-Pei, and Hui-Lin Pan. Potentiation of glutamatergic synaptic input to supraoptic neurons by presynaptic nicotinic receptors. Am J Physiol Regulatory Integrative Comp Physiol 281: R1105–R1113, 2001.—The release of vasopressin and oxytocin from the supraoptic nucleus (SON) neurons is tonically regulated by excitatory glutamatergic and inhibitory GABAergic synaptic inputs. Acetylcholine is known to excite SON neurons and to elicit vasopressin release. Cholinergic receptors are located pre- and postsynaptically in the SON, but their functional significance in the regulation of SON neurons is not fully understood. In this study, we determined the role of presynaptic cholinergic receptors in regulation of the excitatory glutamatergic inputs to the SON neurons. The magnocellular neurons in the rat hypothalamic slices were identified microscopically, and the spontaneous miniature excitatory postsynaptic currents (mEPSCs) were recorded using the whole cell voltage-clamp technique. The mEPSCs were abolished by the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (20 μM). Acetylcholine (100 μM) significantly increased the frequency of mEPSCs of 38 SON neurons from 1.87 ± 0.36 to 3.42 ± 0.54 Hz but did not alter the amplitude (from 19.61 ± 0.90 to 19.34 ± 0.84 pA) and the decay time constant of mEPSCs. Furthermore, the nicotinic receptor antagonist mecamylamine (10 μM, n = 16), but not the muscarinic receptor antagonist atropine (100 μM, n = 12), abolished the excitatory effect of acetylcholine on the frequency of mEPSCs. These data provide new information that the excitatory effect of acetylcholine on the SON neurons is mediated, at least in part, by its effect on presynaptic glutamate release. Activation of presynaptic nicotinic, but not muscarinic, receptors located in the glutamatergic terminals increases the excitatory synaptic input to the SON neurons of the hypothalamus.

vasopressin; cholinergic receptors; muscarinic receptors; glutamate; synapse; magnocellular neurons

The supraoptic nucleus (SON) neurons in the hypothalamus are responsible for the synthesis of vasopressin and oxytocin. The SON neurons project into the neurohypophysis and release these two peptides into the systemic circulation. The pulsatile release of these peptides is controlled by neuronal activity of the SON, which is influenced by body fluid volume, osmolarity, extracellular Na+ concentrations, and blood pressure (10, 18). Synaptic inputs play an important role in regulation of the excitability of SON neurons. Glutamate is the major excitatory neurotransmitter in the central nervous system. Glutamate receptors are located on the presynaptic nerve terminals and postsynaptic neurons in several hypothalamic regions including the SON (4, 33, 35). It has been demonstrated that glutamate acting at ionotropic non-N-methyl-D-aspartate receptors is largely responsible for excitatory synaptic transmission in hypothalamic SON neurons (7, 11, 35, 36). Furthermore, blockade of glutamate receptors inhibits vasopressin release evoked by the hyperosmotic stimulus (28), spontaneous activity of SON neurons (2), and spontaneous and evoked excitatory postsynaptic potentials (7). Many neuromodulators, such as nitric oxide and norepinephrine, can influence vasopressin and oxytocin release indirectly through their actions on the synaptic inputs to the SON (20, 26).

Acetylcholine is important for the regulation of SON neurons of the hypothalamus. For instance, the SON region is rich in acetylcholinesterase (13), choline acetyltransferase (13, 24), and nicotinic receptors (12, 16). Cholinergic neurons are located in a region dorsolateral to the SON (13, 14). Electrical stimulation of this region in the hypothalamic slice activates SON neurons, an effect that is blocked by nicotinic, but not muscarinic, receptor antagonists (8). Local application of exogenous acetylcholine or cholinomimetics also increases the discharge activity of SON neurons in vivo and in vitro (5, 6). Furthermore, acetylcholine evokes vasopressin release from the neurohypophysis when applied to the SON region (29), and the effect of acetylcholine on vasopressin release is mediated by nicotinic, but not muscarinic, receptors in the hypothalamus (5). The cholinergic nicotinic receptors are present at the presynaptic nerve terminals and in the postsynaptic SON neurons (27, 38). However, the sites (pre- vs. postsynaptic) and mechanisms of action of acetylcholine in the regulation of the excitability of the SON neurons remain uncertain.

Using the method of whole cell voltage-clamp recordings in the in vitro hypothalamic slices, it has been documented that the miniature excitatory postsynaptic
current (mEPSC) recorded from the SON neurons directly reflects spontaneous release of glutamate from the terminals of glutamatergic neurons in the SON (11, 37). Because synaptically released glutamate mediates the major excitatory synaptic input to the SON neurons, one of the possible mechanisms of the excitatory effect of acetylcholine on SON neurons could be mediated by its effect on presynaptic glutamate release. Little information is available about the effect of acetylcholine on the glutamatergic synaptic input to the SON neurons. Therefore, in the present study, we tested a hypothesis that acetylcholine potentiates the excitatory glutamatergic synaptic input to the SON neurons through activation of presynaptic nicotinic receptors.

METHODS

Slice preparations. Sprague-Dawley rats (3–5 wk old; Harlan Industries, Indianapolis, IN) were anesthetized with halothane and rapidly decapitated. The brain was quickly removed and placed in ice-cold perfusion solution for 1–2 min. During the dissection, particular care was taken to

Fig. 1. A: photomicrograph of a magnocellular neuron (+) with an attached recording electrode (×) in the fresh tissue slice viewed under a microscope with differential interference contrast optics. Magnification ×600. B: morphology of a magnocellular neuron labeled with biocytin. Magnification ×600.

Fig. 2. Spontaneous mEPSCs of a supraoptic nucleus neuron recorded at a holding potential of −70 mV. Representative traces of miniature excitatory postsynaptic currents (mEPSCs) from a single supraoptic nucleus (SON) neuron during control (A), application of 1 μM TTX (B), perfusion of 100 μM acetylcholine (C), and perfusion of 20 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) + 100 μM acetylcholine (D) are shown.
remove the meninges and to cut the optic nerves without applying any tension on the tissue. A tissue block containing the hypothalamus was cut from the brain and was glued on the stage of the vibratome (Technical Product International, St. Louis, MO). Coronal slices (300 μm thick) containing the SON were cut from the tissue block at 4°C. The slices were then trimmed carefully with a circular punch as described previously (11) and preincubated in the perfusion solution continuously oxygenated with 95% O₂–5% CO₂ at 34°C for ≥1 h until they were transferred to the recording chamber. The perfusion solution contained (in mM) 124.0 NaCl, 3.0 KCl, 1.3 MgSO₄, 2.4 CaCl₂, 1.4 NaH₂PO₄, 10.0 glucose, and 26.0 NaHCO₃. Acetylcholine, TTX, bicuculline methiodide, mecamylamine, atropine, and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (all from Sigma, St. Louis, MO) were prepared immediately before the experiments and applied to the slice preparation using syringe pumps. The surgical preparations and experimental protocols were approved by the Animal Care and Use Committee of the Pennsylvania State University College of Medicine and conformed to the National Institutes of Health guidelines on the ethical use of animals. All efforts were made to minimize the suffering and number of animals used.

Measurements of excitatory postsynaptic currents. Recordings of spontaneous excitatory postsynaptic currents (EPSCs) were performed in a radio frequency-shielded room using the whole cell voltage-clamp method (11, 37). The electrode for the whole cell recordings was triple-pulled with a puller (model P-97, Sutter Instrument, Novato, CA) using borosilicate glass capillaries (1.2 mm OD, 0.86 mm ID; World Precision Instruments, Sarasota, FL). The resistance of the pipette tip was 5–10 MΩ when it was filled with the pipette solution containing (in mM) 130.0 potassium gluconate, 1.0 MgCl₂, 10.0 HEPES, 10.0 EGTA, 1.0 CaCl₂, and 4.0 ATP-Mg at pH 7.25. The slice was placed in a glass-bottomed chamber (Warner Instruments, Hamden, CT) and fixed with a grid of parallel nylon threads supported by a U-shaped stainless steel weight. The slice was perfused at 2.0 ml/min at 34°C maintained by an in-line solution heater and a temperature controller (model TC-324, Warner Instruments). Magnocellular neurons in the SON were identified through a fixed-stage microscope (model BX50WI, Olympus) with Nomarski optics and a water immersion objective (×600) (11). The tissue image was captured and enhanced through a charge-coupled device digital camera (Optronics, Goleta, CA) and displayed on a video monitor (Sony).

Positive pressure was continuously applied to the pipette, which was advanced toward the identified neuron through a motorized manipulator (model MP285, Sutter Instrument) under direct visual control. Once the pipette touched the membrane of the neuron, the pressure was immediately released and slight negative pressure was applied to establish a high-resistance seal. The cell membrane was then ruptured by further suction to record in the whole cell configuration. Recordings of postsynaptic currents began ~5 min after the whole cell access was established and the current reached a steady state. EPSCs were recorded using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA) at a holding potential of −70 mV (11, 37). Signals were filtered at 1–2 kHz, digitized at 10 kHz (DigiData 1320A, Axon Instruments), and stored into a Pentium computer using the pCLAMP 8.01 program (Axon Instruments). The postsynaptic currents were analyzed off-line with a peak detection program (Minianalysis, Synaptosoft, Leonia, NJ). All spontaneous mEPSCs were recorded in the presence of TTX (1 μM) and bicuculline (20 μM) unless otherwise stated. To ensure that the recorded cells were located in the SON, some cells were recorded with pipettes containing 0.4% biocytin. At the end of the experiment, the slice containing the labeled cell was immersed in 4% paraformaldehyde and fixed for several days. The tissue was then cut at 50 μm on a freezing microtome (Leica) and rehydrated. The sections were stained with an avidin-biotinylated horseradish peroxidase, as described previously (9).

Experimental protocols. After the whole cell recordings were established, the holding potential was set to the resting membrane potential and maintained for ≥5 min to obtain a stable recording. The resting potential and the input resistance were monitored throughout the recording period. Data were excluded if the input resistance changed >15%. After the mEPSCs were recorded for 5 min as the baseline control, 100 μM (final concentration) acetylcholine was perfused into the slice for 3–5 min. In 10 SON neurons, the reproducible effect of acetylcholine on mEPSCs was determined 15–20 min after initial acetylcholine perfusion and the baseline mEPSCs had returned to the control. In 8 of these 10 SON neurons, we also tested the effect of 20 μM CNQX on the spontaneous mEPSCs and the effect of acetylcholine on mEPSCs in the presence of CNQX. To determine the role of muscarinic and nicotinic receptors in acetylcholine-elicited changes in the mEPSCs of SON neurons, 10 μM mecamylamine

Fig. 3. Repeatability of responses of mEPSCs of SON neurons to bath application of acetylcholine. Application of 100 μM acetylcholine produced reproducible changes in amplitude (A) and frequency (B) of mEPSCs of SON neurons. Values are means ± SE (n = 10). *P < 0.05 compared with control (Kruskal-Wallis ANOVA followed by Dunn’s post hoc test).
(n = 16), a neuronal nicotinic receptor antagonist (17), or 100 μM atropine (n = 12), a muscarinic receptor antagonist (17), was applied together with acetylcholine 15–20 min after the initial effect of acetylcholine on mEPSCs was tested. The effective concentrations of acetylcholine and the two cholinergic receptor antagonists have been determined in previous studies (5, 6, 17). In addition, to determine whether the presence of inhibitory postsynaptic currents altered the effect of acetylcholine on mEPSCs, the above experiments were repeated in a separate group of neurons (n = 16) in which the spontaneous mEPSCs were recorded in the absence of bicuculline.

Values are means ± SE. The analysis of mEPSCs was performed with the cumulative probability plot (31). The cumulative probability of the amplitude and interevent interval was compared by the Komogorov-Smirnov test (Minianalysis), which estimates the probability that two cumulative distributions are similar. The effects of drugs on the amplitude and frequency of mEPSCs were determined by the nonparametric (Wilcoxon signed rank) test or nonparametric ANOVA (Kruskal-Wallis) test with Dunn’s post hoc test. P < 0.05 was considered to be statistically significant.

RESULTS

Effect of acetylcholine on spontaneous mEPSCs of SON neurons. Spontaneous EPSCs were recorded from a total of 38 magnocellular neurons (n = 32 rats) in the SON that were identified microscopically in the slice preparation (Fig. 1A). We recovered 12 SON neurons labeled with biocytin (Fig. 1B), and all were located within the boundary of the SON. Once the whole cell recording was established, stable recordings were usually achieved for 2–3 h without noticeable change in the resting membrane potential or input resistance. The mean resting membrane potential was −57.49 ± 2.02 mV, and the mean input resistance was 824 ± 52 MΩ. The amplitude of mEPSCs recorded ranged from 10 to 150 pA (19.61 ± 0.90 pA), and the frequency ranged from 0.5 to 6 Hz (1.87 ± 0.36 Hz, n = 38). The amplitude and frequency of mEPSCs of 16 additional SON neurons recorded in the absence of bicuculline were 18.5 ± 0.94 pA and 1.98 ± 0.33 Hz, respectively, which were similar to values observed in the presence of 20 μM bicuculline. Perfusion of 1 μM TTX had little...

Fig. 4. Inhibitory effect of mecamylamine on the response of mEPSCs of an SON neuron to acetylcholine. Representative records show mEPSCs during control (A), application of 100 μM acetylcholine (B), and application of 10 μM mecamylamine (MCM) + acetylcholine (C). Cumulative plot analysis of mEPSCs of the same SON neuron showing the distribution of the peak amplitude (D) and the interevent interval (E) are also shown. F: superimposed averages of 100 consecutive mEPSCs obtained during control and acetylcholine application. Dashed line, single-exponential fit to the control average. Note similar amplitude of both averages (20.22 vs. 21.10 pA) and similar time decay constants (r = 1.52 ms).

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effect on spontaneous mEPSCs of 38 SON neurons (Fig. 2B). Bath perfusion of 20 μM CNQX completely abolished spontaneous mEPSCs, and reapplication of acetylcholine did not induce mEPSCs of eight SON neurons in the presence of CNQX (Fig. 2D).

Application of 100 μM acetylcholine reversibly increased the frequency of mEPSCs from 1.87 ± 0.36 to 3.42 ± 0.54 Hz (P < 0.05, n = 38). However, the amplitude of mEPSCs was not affected significantly by acetylcholine (Figs. 2 and 3). The effect of acetylcholine on the frequency of mEPSCs is reproducible (Fig. 3, n = 10). The cumulative probability analysis of mEPSCs revealed that the distribution pattern of the inter-event interval shifted leftward in response to acetylcholine, but the distribution pattern of the amplitude was not affected by acetylcholine (Fig. 4). The effect of acetylcholine on mEPSCs was further analyzed by measuring the time constant of the decay phase of the spontaneous mEPSCs by single-exponential curve fitting. The decay phase of mEPSCs was generally well fitted by a single-exponential curve (Fig. 4). The averaged peak amplitude (19.79 ± 1.05 vs. 21.29 ± 1.42 pA, P > 0.05) and the decay time constant (1.56 ± 0.24 vs. 1.49 ± 0.21 ms, P > 0.05) obtained during acetylcholine application were not significantly different from the control. In the absence of bicuculline, the frequency of the mEPSCs increased from 1.87 ± 0.36 to 3.42 ± 0.54 Hz (n = 16, P < 0.05) during the application of acetylcholine, while the amplitude of mEPSCs was not changed. These data were similar to mEPSCs of SON neurons recorded in the presence of CNQX during control (1.59 ± 0.48 vs. 1.97 ± 0.37 Hz and 21.29 ± 1.42 pA, respectively, during mecamylamine application; Figs. 2 and 3). In the presence of CNQX, application of 100 μM acetylcholine failed to in-duce mEPSCs of eight SON neurons. Changes of the amplitude (A) and frequency (B) of mEPSCs of SON neurons during control, application of 100 μM acetylcholine, and application of acetylcholine plus 10 μM mecamylamine are shown. Values are means ± SE (n = 16). *P < 0.05 compared with control (Kruskal-Wallis ANOVA followed by Dunn’s post hoc test).

Role of nicotinic cholinergic receptors in the effect of acetylcholine on mEPSCs. To study the role of nicotinic receptors in the excitatory effect of acetylcholine on mEPSCs, the selective nicotinic receptor antagonist mecamylamine (10 μM) was used. Bath perfusion of mecamylamine alone for 5 min had no effect on the frequency and amplitude of mEPSCs. The frequency and amplitude of mEPSCs were 1.97 ± 0.37 Hz and 19.65 ± 0.78 pA, respectively, during mecamylamine perfusion. Using the cumulative probability analysis, we found that acetylcholine shifted the distribution of the interevent interval leftward without changing the distribution of the amplitude or kinetics of mEPSCs, indicating that acetylcholine selectively increased the frequency of mEPSCs (Figs. 4 and 5). Mecamylamine abolished the excitatory effect of acetylcholine on the frequency of mEPSCs (Fig. 5). The distribution pattern of the amplitude and the interevent interval were not changed by the application of acetylcholine in the presence of mecamylamine (Fig. 4). In the presence of mecamylamine, 100 μM acetylcholine failed to increase the frequency of mEPSCs (Fig. 5; n = 16). During application of acetylcholine, the time constant of the decay phase and the averaged peak amplitude of the mEPSCs were 1.48 ± 0.21 ms and 21.29 ± 1.42 pA, respectively. These data were not statistically different from those during control (1.59 ± 0.35 ms and 20.38 ± 2.01 pA, P > 0.05; Fig. 4). In the absence of bicuculline, the frequency and the amplitude of mEPSCs recorded during control (1.84 ± 0.62 Hz and 20.38 ± 2.32 pA) were not significantly altered by the application of acetylcholine plus mecamylamine (1.98 ± 0.51 Hz and 19.34 ± 3.02 pA, P > 0.05, n = 7). The inhibitory effect of mecamylamine on the action of acetylcholine on mEPSCs of SON neurons was not affected in the absence of bicuculline (data not shown).

Role of muscarinic receptors in the effect of acetylcholine on mEPSCs. Perfusion with 100 μM atropine alone did not affect the amplitude and the frequency of mEPSCs. In the presence of atropine, application of 100 μM acetylcholine still significantly increased the frequency of mEPSCs but did not change the amplitude of mEPSCs compared with the controls (P < 0.05, n = 12; Figs. 6 and 7). The cumulative probability analysis of mEPSCs indicated that the distribution pattern of the interevent interval shifted leftward, while the amplitude distribution was not changed in response to acetylcholine or acetylcholine plus atropine (Fig. 6). The averaged peak amplitude (19.35 ± 0.61 pA) and the decay time constant (1.67 ± 0.26 ms) of mEPSCs obtained during acetylcholine application were not significantly different from the values obtained...
with acetylcholine plus atropine (18.59 ± 0.43 pA and 1.78 ± 0.48 ms; Fig. 6). In the absence of bicuculline, the frequency of mEPSCs was also significantly increased from 1.92 ± 0.48 Hz during control to 3.89 ± 0.52 Hz during application of acetylcholine, while the amplitude of mEPSCs was not changed significantly (from 18.40 ± 1.56 to 19.65 ± 2.12 pA, P > 0.05, n = 8).

DISCUSSION

This is the first study demonstrating the functional significance of presynaptic nicotinic receptors at the terminals of glutamatergic neurons in the regulation of the excitability of SON neurons. Although acetylcholine is known to excite SON neurons and induce vasopressin release, the presynaptic effect of acetylcholine and the cholinergic receptor types involved in the synaptic release of glutamate in the SON neurons have not been investigated previously. There are two new findings in the present study. First, we found that acetylcholine significantly increased the frequency, but not the amplitude, of mEPSCs of SON neurons. Furthermore, the excitatory effect of acetylcholine on the frequency of mEPSCs of SON neurons was blocked by mecamylamine, but not by atropine. Because glutamatergic neurons constitute the major excitatory synaptic inputs into the SON, an increase in mEPSCs would potentiate the excitability of postsynaptic SON neurons. Thus these data suggest that presynaptic nicotinic cholinergic receptors play an important role in the regulation of glutamatergic inputs to the SON neurons and the control of vasopressin and oxytocin secretion.

The SON is a hypothalamic structure that contains predominantly neurosecretory neurons that synthesize, store, and release vasopressin and oxytocin. The SON neurons project to the neurohypophysis, where vasopressin and oxytocin are released into the general circulation to influence physiological functions such as fluid balance, cardiovascular regulation, parturition, and lactation (25). Secretion of these two peptides is strongly influenced by the afferent input and neuronal activity of SON neurons. The major afferent signals to the SON are those from excitatory glutamatergic inputs and inhibitory GABAergic inputs (11, 26, 37). Presynaptic glutamatergic and GABAergic neurons innervate SON neurons (32). The glutamate receptors and the mRNA for glutamate receptors are present in
post hoc test). Many factors such as the study design and experiment conditions exist (23). Cholinergic neurons are located dorsolateral to the SON and send processes directly into the SON region (34). Because all mEPSCs recorded from SON neurons are blocked by CNQX, the mEPSCs represent the quantal release of glutamate from the presynaptic terminals (11, 37). Previous studies have shown that the presence of functional synaptic boutons attached to the postsynaptic cell is a sufficient condition for miniature spontaneous activity to occur, and the cell bodies and axons of the projecting cells are not essential (3, 37). By recording the miniature spontaneous activity in the slice using the whole cell voltage-clamp technique, it is possible to study the synaptic inputs to a given cell. With this approach, it has been shown that many endogenous neuromodulators, including nitric oxide, norepinephrine, and prostaglandins, can act at the presynaptic sites to affect synaptic transmission in the SON (11, 20, 26).

Acetylcholine is an important endogenous neuromodulator regulating SON neurons. In this regard, choline acetyltransferase-positive neurons are found in the dorsolateral boundaries of the SON (14, 32). Acetylcholinesterase activity has been demonstrated in SON neurons, where many vasopressin-secreting neurons exist (23). Cholinergic neurons are located dorsolateral to the SON and send processes directly into the SON through a monosynaptic pathway (8, 13, 30). Thus cholinergic neurons could regulate vasopressin neurons through a synaptic mechanism. In the present study, we found that acetylcholine increased the frequency of mEPSCs without an effect on the amplitude and kinetics of mEPSCs, indicating that the effect of acetylcholine is mediated by presynaptic nicotinic receptors located at terminals of glutamatergic neurons in the SON. This observation suggests that the site of some excitatory effects of acetylcholine on SON neurons is, at least in part, at the presynaptic terminals of glutamatergic neurons.

Muscarinic and nicotinic receptors are located within the hypothalameurohypophysial system (15). It has been shown that microinjection of nicotinic, but not muscarinic, receptor agonists into the SON stimulates vasopressin release (19). We found that the excitatory effect of acetylcholine on mEPSCs of SON neurons was blocked by mecamylamine, but not by atropine. These data suggest that nicotinic receptors located at the presynaptic terminals of glutamatergic neurons likely play an important role in the regulation of vasopressin release. Our finding is consistent with many previous studies showing that acetylcholine elicits vasopressin synthesis and release through nicotinic, but not muscarinic, receptors in the SON (6, 19, 29). In this regard, nicotinic receptors have been detected in presynaptic and postsynaptic nerve terminals in the SON (22, 27, 38). Also, local application of nicotine excites SON neurons in hypothalamic slices (6), and nicotinic receptor antagonists effectively block vasopressin release caused by osmotic changes in organ-cultured hypothalamic explants (5, 29). We found that perfusion with mecamylamine alone had no effect on the spontaneous mEPSCs, suggesting no spontaneous release of acetylcholine in this “punch-out” slice preparation. Taken together, the results from the present study support the notion that acetylcholine is capable of increasing the excitatory glutamatergic inputs to the SON neurons through activation of presynaptic nicotinic receptors and, subsequently, causes excitation of magnocellular neurons within the SON to evoke vasopressin/oxytocin release. We also observed that the effect of acetylcholine on mEPSCs in the presence of bicuculline was similar to that without bicuculline. These data suggest that the effect of acetylcholine on the mEPSCs is independent of the inhibitory GABAergic inputs to the SON neurons.

It should be acknowledged that there are at least two different subtypes of cholinergic nicotinic receptors located pre- and postsynaptically in the SON: α4- and α7-containing nicotinic receptors (27, 38). Mecamylamine used in this study is capable of blocking both subtypes of nicotinic receptors. A recent study has shown that acetylcholine produces a rapid inward current in the SON neurons through activation of α7-containing nicotinic receptors (38). It is not entirely clear why we only observed a presynaptic effect of acetylcholine on SON neurons in this study. Many factors such as the study design and experi-

Fig. 7. Effect of atropine on acetylcholine-elicited changes in mEPSCs of SON neurons. Changes of the amplitude (A) and frequency (B) of mEPSCs of SON neurons are shown during control, application of 100 μM of acetylcholine, and application of acetylcholine + 100 μM atropine. Values are means ± SE (n = 12). *P < 0.05 compared with control (Kruskal-Wallis ANOVA followed by Dunn’s post hoc test).
mental preparations may contribute to the discrepancy. For example, spontaneous mEPSCs were recorded in our study to assess the effect of acetylcholine perfused for 3–5 min on glutamate release in the hypothalamic slice maintained at 34°C. In the report by Zaninetti et al. (38), acetylcholine was applied only for 100–200 ms, with fast pressure ejection to the dissociated SON neurons and the hypothalamic slice (at room temperature) used to record the evoked current. The authors did not examine the effect of acetylcholine on spontaneous mEPSCs of SON neurons in their study. Most importantly, it has been demonstrated that the evoked currents by activation of α7-containing nicotinic receptors decay close to baseline values within 100–1,000 ms during acetylcholine application (1, 21). Thus the lack of a postsynaptic effect of acetylcholine in the present study is likely due to the bath application of acetylcholine rapidly desensitizing the α7-nicotinic receptors in the hypothalamic slice (38).

In summary, this study indicates that acetylcholine increases glutamate release in the SON neurons through activation of presynaptic nicotinic receptors. Such a presynaptic mechanism could play an important role in the excitatory control of the SON neurons by cholinergic afferent nerves. Therefore, activation of presynaptic nicotinic receptors is important, at least in part, for the cholinergic regulation of the excitatory glutamatergic input to the SON neurons, which further influences the release of vasopressin and oxytocin.

**Perspectives**

Previous studies have shown that endogenous acetylcholine can excite SON neurons and cause release of vasopressin and oxytocin. Because an increase in mEPSCs reflects the spontaneous release of more vesicles containing glutamate, activation of presynaptic nicotinic receptors could increase the excitation of postsynaptic SON neurons. We have demonstrated that one of the potential mechanisms of the action of acetylcholine on SON neurons is to increase their excitability through augmented synaptic glutamate release. The data from the present study suggest that non-α7-nicotinic receptors may be involved in the control of glutamatergic synaptic inputs to SON neurons. The role of nicotinic receptor subunits other than the α7-type responsible for the presynaptic effect of acetylcholine on SON neurons warrants further studies. Furthermore, the excitability of SON neurons is also strongly influenced by the inhibitory GABAergic synaptic input (11, 37). The effect of acetylcholine on synaptic GABA release in SON neurons has not been determined previously. Finally, many neurotransmitters/neuromodulators such as nitric oxide, norepinephrine, and prostaglandins can affect glutamate and GABA release (20, 26). Future studies are needed to determine their interactions with acetylcholine in the synaptic regulation of the activity of SON neurons under physiological and pathophysiological conditions.

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