Histamine Suppresses Non-NMDA Excitatory Synaptic Currents in Rat Supraoptic Nucleus Neurons

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INTRODUCTION

Histamine (HA) has been proposed to function both as a transmitter and a modulator in the brain (Hatton and Li 1998a; Prell and Green 1986). Released from presynaptic terminals in response to electrical stimulation of the tuberomammillary nucleus (TM) or to dehydration (Akins and Bealer 1993; Kjaer et al. 1994), HA not only elicits excitatory or inhibitory postsynaptic potentials (Weiss et al. 1989; Yang and Hatton 1994) but also regulates gap junctional communication (Hatton and Yang 1996). The latter, as well as HA-induced prolonged depolarization and enhancement of depolarizing after potentials (DAPs) (Smith and Armstrong 1993; Yang and Hatton 1989), is mediated by activation of various intracellular messenger systems (Leurs et al. 1995). Our recent experiments demonstrated that prevention of G proteins, phospholipase C (PLC) or protein kinase C (PKC) from activation blocks HA-induced depolarization and enhancement of currents underlying depolarizing after potentials (IDAP) (Li and Hatton 1996; Li et al. 1999). Reduced nitric oxide (NO) production with inhibitors of NO synthase (NOS) cancels HA-induced increases in dye coupling among supraoptic nucleus (SON) neurons (Yang and Hatton 1999).

The SON receives rich glutamatergic inputs that are critical in the regulation of hypothalamic neuroendocrine activity (Armstrong 1995; Hatton 1990; van den Pol et al. 1990). Glutamate can bind to postsynaptic non-N-methyl-D-aspartate (NMDA), mainly AMPA, and NMDA receptors to accomplish neurotransmission (Mayer and Westbrook 1987; Ozawa et al. 1998). Activation of ionotropic glutamate receptors usually causes membrane depolarization and burst activities in SON neurons, resulting in increased release of both vasopressin and oxytocin (Gribkoff and Dudek 1990; Hu and Bourque 1992; Saybasili et al. 1995). Because HA is known to modulate excitatory postsynaptic currents (EPSCs) in hippocampal neurons (Bekkers 1993; Brown et al. 1995; Nikmanesh et al. 1996; Saybasili et al. 1995), here we examined whether HA influences non-NMDA glutamate receptor-mediated synaptic currents in SON neurons and explored possible intracellular mechanisms involved. For comparison, we also tested the effects of norepinephrine (NE) on synaptic currents. NE is another neurotransmitter that is reported to activate intracellular signal transduction systems similar to those used by HA (Armstrong 1995; Minneman and Esbenshade 1986). The results showed that HA reversibly suppresses non-NMDA EPSCs via activating H1 receptors, and NO is partially responsible for these actions of HA. A preliminary report of this study has appeared in abstract form (Hatton and Li 1998b).
METHODS

Preparation

Male rats, 50–70 days, were decapitated using a rodent guillotine. Their brains were removed, and horizontal slices of the hypothalamus (250- to 300-μm thick) were cut using a vibratome. Oxygenated (95% O₂-5% CO₂) artificial cerebrospinal fluid (ACSF) contained (mM) 126 NaCl, 5 KCl, 1.3 NaHPO₄, 2.4 CaCl₂, 3.0, MgSO₄, 26 NaHCO₃, 10 glucose, and 5 3(N-morpholino)propanesulfonic acid buffer (310 mOsml/l, pH 7.4). After incubation at room temperature (23°C) for >2 h, the slices were transferred to a submerging recording chamber and perfused with warmed ACSF (36°C) at 2 ml/min. To abolish GABAergic inhibitory synaptic inputs, bicuculline (10–15 μM) was added to the ACSF.

Electrophysiological recordings

Patch electrodes were pulled from borosilicate capillary tubing and filled with the following solution (mM): 140 K⁺ gluconate, 2 MgCl₂,10 HEPES, 2 K₂ATP, and 0.4 Na₂GTP (pH 7.25). Electrodes usually had outer tip diameters of ~2.7 μm and DC resistances of 4–5 MΩ. Whole cell patch-clamp recordings were obtained from SON neurons using an Axoclamp-2B amplifier (Axon Instruments, Foster City, CA). Patch electrodes approached the SON under visual guidance using a dissecting microscope. Whole cell recordings were obtained using current-clamp techniques. Hyperpolarizing currents were repeatedly passed to induce voltage responses that increased in amplitude when electrode tips touched neuronal membranes. Gigahm seals between electrodes and cell membranes then were obtained by applying gentle suction. When a seal was achieved, further brief suction was applied to break through the membrane. Whole cell recordings were indicated by observation of negative membrane potentials and action potentials. KCl/agar bridges were used as reference electrodes and correction of liquid junction potential (~7 mV) was applied to recorded membrane voltages.

Continuous single-electrode voltage-clamp experiments were performed to investigate the EPSCs. Cells were clamped around ~70 mV, and amplifier feedback gain was set at 40–90 nA/mV and low-pass filter at 1.0 kHz. Series resistance was evaluated by measuring instantaneous currents evoked by a hyperpolarizing pulse (10 mV, 40 ms) and ranged from 4 to 10 MΩ. Membrane conductance was monitored during the experiment using hyperpolarizing pulses (~10 mV, 75 ms). Electrical stimulation was delivered through a concentric electrode positioned in an area immediately lateral to the posterior SON (Fig. 1A). Current intensity was increased gradually until EPSCs were evoked by each stimulus (0.2 ms, 50–300 μA). Once per minute, four EPSCs at an interval of 5 s were acquired consecutively and averaged. EPSC duration is the period from the beginning of stimulating pulses to the return of the inward current to baseline, whereas EPSC amplitude refers to the maximal value of the inward current compared with baseline. The change in EPSC was determined by comparing ion currents obtained before and 1, 3, 5, and 10 min after perfusion of slices with test agents. Data acquisition and analyses were performed using a computer operating AXOTAPE and pCLAMP (Axon Instruments). All data are presented as means ± SE and repeated measures ANOVA was applied for statistical analyses.

Chemicals

HA, NE, pyrilamine (H₁ receptor antagonist), dimaprit (H₂ receptor agonist), imetit (H₃ receptor agonist, Tocris, Ballwin, MO), and N⁶-nitro-L-arginine methyl ester (L-NAME) were stored in concentrated solutions (10⁻⁴–10⁻² M) and diluted into ACSF before experiments. 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, RBI, Natick, MA), 2-amino-5-phosphonovaleric acid (APV), and 8-bromo-queuosine 3',5'-cyclic monophosphate (8-bromo-cGMP) were directly dissolved in ACSF and applied. When needed, guanosine 5'-O-(2-thiodiphosphate) (GDP-β-S), 1-(5-isouquinolinylsulfonyl)-2-methyl-piperazine (H₇), and 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) were added to the electrode filling solution and applied intracellularly after membrane rupture. Except where indicated, test agents were usually bolus-injected into perfusion pipeline. It took ~30 s before test agents reached the recording chamber, after which the effects of agents were observed. Because of prolonged effects on SON neurons of activating intracellular second-messenger systems (Armstrong and Sladek 1985; Li and Hatton 1996; Li et al. 1999), such brief exposure of SON cells to HA and NE, provided by bolus injections, achieves a much better recovery rate than bath application. The final concentrations of HA and NE in the recording chamber were ~10 μM (50 times dilution). Except as otherwise stated, all chemicals used were purchased from Sigma, St. Louis, MO.

Animal care and use during this study were in accordance with National Institutes of Health and institutional guidelines and policy on the use of animals in research. All efforts were made to minimize animal suffering and to reduce the number of animals used.

RESULTS

Evoked non-NMDA EPSCs in supraoptic nucleus neurons

Whole cell patch-clamp recordings were obtained from 68 SON neurons. The resting membrane potentials ranged from ~55 to ~75 mV, amplitudes of action potentials from 70 to 100 μV, and membrane input resistances from 0.2 to 0.8 GΩ. In bridge mode without DC current injection, 38% of these cells displayed phasic patterns of firing, with DAPs following EPSPs the amplitudes and durations of which were dependent on the level of the membrane potential.

FIG. 1. A: diagram of horizontally cut brain slice illustrating electrical stimulation (Sti) and patch recording (Rec) sites. 3V, third ventricle; LOT, lateral olfactory tract; OT, optic tract; SON, supraoptic nucleus; TM, tube-romammillary nucleus. B: electrical stimulation (↓, 0.2 ms, 100 μA) evoked excitatory postsynaptic potentials the amplitudes and durations of which were dependent on the membrane potential.

By 10.220.32.246 on September 27, 2016
In SON neurons showing evoked EPSPs, voltage-clamp experiments were carried out and inward postsynaptic currents, i.e., EPSCs, were induced after brief electrical stimulation (Fig. 2A). When cells were voltage-clamped at −70 mV, evoked EPSCs had amplitudes of 88.4 ± 9.6 pA (n = 43). Their rise times ranged from 2–7 ms and durations were 41.1 ± 3.0 ms. With twin stimulus pulses used (20 Hz repeated every 5 s), EPSCs evoked by the second pulse (171.2 ± 11.5 pA, 63.2 ± 4.8 ms; n = 43) were always larger than those by the first pulse (for the amplitude and duration, P < 0.001 and <0.05, respectively). Mean paired-pulse facilitation (PPF) ratios were 1.93 ± 0.12. Bath application of 10–20 μM CNQX, a non-NMDA receptor antagonist, for 2 min abolished EPSCs (Fig. 2B). CNQX did not change the PPF significantly (from 1.91 ± 0.10 in control to 1.64 ± 0.26, P > 0.05). After bath perfusion of slices with 50 μM APV for 10 min, however, EPSCs decreased by −10% (n = 3). These data suggest that EPSCs recorded in the current experiments are predominately gated by non-NMDA (AMPA/kainate) glutamate receptors.

**Suppression of EPSCs by histamine**

HA effectively suppressed EPSCs in all 15 SON neurons tested (Fig. 3). These cells displayed phasic or nonphasic patterns of firing, with or without DAPs. Within 2 min after HA application, EPSC amplitudes and durations decreased by 61.5 and 31.0%, respectively (from 67.5 ± 12.7 pA and 44.9 ± 4.8 ms in control to 26.0 ± 6.4 pA to 31.0 ± 5.4 ms, n = 15; P < 0.001 for both). Similarly, HA suppressed EPSCs evoked by the second pulses. The amplitudes and durations decreased by 59.4 and 27.5%, resulting in no change in PPF (from 1.87 ± 0.13 in control to 1.95 ± 0.14, n = 15; P > 0.05). Recovery of EPSCs from HA suppression was readily observed. EPSC amplitudes were 66.8, 86.7, and 95.6% of the control at 3, 5, and 10 min after HA application, whereas EPSC durations were 83.7, 95.6, and 108.5% (n = 15), respectively. Because EPSCs evoked by the second pulses recovered faster, the PPF rose to 118.2 and 126.2% of the control (1.87 ± 0.13, n = 15) at 3 and 5 min after HA application, although these differences were not statistically significant (P > 0.05). We examined the dose-response relationship in another five cells, and bath perfusion of HA at concentrations of 0.1, 1, and 10 μM suppressed EPSC amplitudes by 21.2, 47.6, and 70.2%, respectively (data not shown).

In contrast to HA, neither dimaprit nor imetit attenuated EPSCs (Fig. 4). Within 2 min after bolus injection of dimaprit, both EPSC amplitudes durations changed little (from 95.6 ±...
12.9 pA and 41.1 ± 6.6 ms in control to 103.7 ± 22.1 pA and 40.5 ± 7.9 ms, n = 5; P > 0.05 for both). There was also no significant difference in PPF (1.65 ± 0.17 in control to 1.53 ± 0.22; P > 0.05). EPSC amplitudes and durations were 69.9 ± 8.3 pA and 44.2 ± 4.9 ms in control, and 77.7 ± 17.2 pA and 41.6 ± 5.8 ms within 2 min after imetit injection (n = 7), which are not significantly different (P > 0.05). The PPF was 1.95 ± 0.17 in control and 2.06 ± 0.23 within 2 min after imetit application (P > 0.05).

After bath application of 10–20 μM pyrilamine for 5 min, HA induced little change in EPSCs (Fig. 4A). EPSC amplitudes were 76.6 ± 9.7 pA in control and 70.6 ± 8.2 pA within 2 min after HA injection (n = 8; P > 0.05), and durations were 38.4 ± 5.6 ms and 42.3 ± 5.4 ms (P > 0.05, respectively). Similarly, the difference in PPF was also not significant (from 1.56 ± 0.11 to 1.62 ± 0.10; P > 0.05). These data suggest that HA suppress EPSCs in SON neurons by activating membrane receptors of the H4 subtype. NE also attenuated EPSCs; these results are described further in a later section.

**Effects of histamine after the blockade of intracellular signal transduction in neurons**

Histaminergic fibers and H4 receptors are abundant in the SON, and their distribution patterns suggest that locally released HA can affect not only SON neurons but also other elements such as glia and presynaptic terminals (Inagaki et al. 1988; Kjaer et al. 1994). The suppression of EPSCs might be mediated by a direct action of HA at SON neurons from which the EPSCs were recorded, by neuromodulators released from neighboring cells after HA application, or by a decrease in transmitter release resulting from the effects of HA and/or neuromodulators on the presynapses. Although the PPF did not increase so greatly as to reach a statistically significant level during HA application, there is still a possibility that presynaptic modulation is partially responsible for EPSC suppression induced by HA. We performed further experiments in the current study, therefore to explore the mechanisms underlying EPSCs suppression.

When patch electrodes containing 0.5 mM GDP-β-S, a nonhydrolysable GDP analogue, were used to record cells, EPSCs recorded were 145.0 ± 31.1 pA and 68.3 ± 4.1 ms (n = 4), which are not significantly different from EPSCs in control (without GDP-β-S; P > 0.05; Fig. 5). Within 2 min after HA injection, EPSCs were 73.8 ± 23.0 pA and 39.7 ± 3.3 ms, i.e., reductions of 49.1 and 41.9% in amplitude and duration (P < 0.05 for both). The PPF went from 1.37 ± 0.17 in control to 1.76 ± 0.62 (P > 0.05). Intracellular diffusion of (10 mM) BAPTA to chelate intracellular-free Ca2+, and (0.5 mM) H7 to antagonize PKC activity, did not abolish HA-

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**Fig. 4.** HA-induced reduction in EPSC amplitudes was prevented by bath perifusion of the H1 receptor antagonist, pyrilamine (Pyri., 10 μM; A). This HA effect was not mimicked by H2 (Dima, dimaprit; 10 μM) or H3 (Imet, Imetit; 10 μM) receptor agonists (B). ● pyrilamine application. ▲ and ↑, when HA and other agonists were bolus-injected, respectively. Note that norepinephrine (NE) also suppressed EPSCs in those neurons. See Fig. 2 for explanation of traces.
induced suppression of EPSCs, either (Fig. 6). In 11 SON neurons recorded using electrodes containing BAPTA, EPSCs were $132.9 \pm 41.2$ pA and $38.6 \pm 5.2$ ms in control and $67.4 \pm 24.9$ pA and $18.6 \pm 4.7$ ms 2 min after HA injection, representing suppression by 49.3 and 51.8% in EPSC amplitude and duration, respectively. The change in PPF from $1.79 \pm 0.16$ in control to $2.05 \pm 0.26$ was nonsignificant (14.5%; $P > 0.05$).

When both BAPTA and H$_7$ were diffused into the cells, HA reduced EPSC amplitudes by 43.9% (from $129.7 \pm 10.2$ pA to $72.8 \pm 23.1$ pA; $P < 0.01$) and durations by 38.4% (from $59.4 \pm 2.6$ ms to $36.6 \pm 10.4$ ms; $P < 0.05$). The PPF did not change significantly: $1.72 \pm 0.13$ in control to $2.19 \pm 0.46$ 2 min after HA application ($P > 0.05$). Again, EPSCs recorded using electrodes containing BAPTA and H$_7$ were not statistically different from those in control (without BAPTA and H$_7$; $P > 0.05$). These results suggest that a direct action of HA at SON neurons recorded using patch electrodes is not the primary mechanism underlying EPSC suppression. Rather it seems likely that this effect is indirect and that HA first acts at SON cells (including neurons and nonneurons), resulting in the production of NO, which then induces postsynaptic changes in the SON neurons recorded.

**Involvement of NO**

Our previous studies have demonstrated that binding of HA to H$_1$ receptors activates an intracellular signal transduction pathway including G-protein, PLC, inositol 1,4,5-trisphosphate, Ca$^{2+}$ release from internal stores, and PKC (Li and Hatton 1996; Li et al. 1999). Ca$^{2+}$-calmodulin complex and PKC can further stimulate NOS and increase production of NO and cGMP (Schuman and Madison 1994). As a result, HA induces prolonged membrane depolarization, enhances DAPs, and facilitates dye transfer among SON neurons (Armstrong and Sladek 1985; Hatton and Yang 1996; Smith and Armstrong 1993). To investigate whether NO diffusion from neighboring neurons is a potential mechanism for EPSC suppression, we carried out further experiments using cGMP and L-NAME.

Bath perifusion of 1–2 mM 8-bromo-cGMP for 5 min reduced EPSC amplitudes by 32.3% (85.7 ± 17.8 pA in control to $58.0 \pm 16.1$ pA; $n = 4$; $P < 0.01$) and durations by 27.9% (from $46.3 \pm 7.1$ to $33.4 \pm 9.5$ ms; $P < 0.05$; Fig. 7). There was no change in PPF (from $1.63 \pm 0.24$ to $2.15 \pm 0.65$; $P > 0.05$). 90% recovery in EPSC amplitude and duration was observed after washout for 10 min. After bath perifusion of slices with 20–25 mM L-NAME, a NOS inhibitor, for 5 min,
HA injection decreased EPSC amplitudes only by 31.9% (from 65.0 ± 3.8 pA in control to 34.3 ± 5.0 pA 2 min after HA application, n = 9; P < 0.05; Fig. 8A). This attenuation was much less than that in control (61.5%; P < 0.01, Student’s t-test). In addition, no change in EPSC durations (from 43.6 ± 4.4 to 36.1 ± 5.2 ms; P > 0.05) or in PPF (1.83 ± 0.13 to 1.90 ± 0.28; P > 0.05) were observed, suggesting a partial blockade of HA’s effect by L-NAME. In SON neurons re-
corded using electrodes containing 10 mM BAPTA and per-
 fused with L-NAMe, the effects of HA on the EPSCs were
 abolished completely (Fig. 8B). The EPSC amplitudes were
 119.8 ± 21.8 pA in control and 101.3 ± 27.8 pA within 2 min
 after HA injection (n = 4; P > 0.05), whereas durations were
 43.3 ± 9.3 and 43.3 ± 6.0 ms, respectively (P > 0.05). The
 PPF was 1.61 ± 0.25 and 1.48 ± 0.21 before and 2 min after
 HA application (P > 0.05).

Comparison with NE

The SON receives dense noradrenergic innervation from the
brain stem nuclei (Armstrong 1995; Hatton 1990). Binding to
 corresponding receptors in the membrane, NE activates intra-
cellular signal pathways, many of which also are involved in
mediating the effects of HA on SON neurons (Leurs et al. 1995).
A recent report has demonstrated that NE can suppress inhibi-
 tory postsynaptic currents (IPSCs) through a presynaptic
mechanism (Wang et al. 1998). Therefore we examined
whether NE affects EPSCs in SON neurons and compared the
effects of NE with those of HA.

The EPSCs, with amplitudes of 72.3 ± 9.4 pA and durations
of 34.6 ± 7.1 ms, were obtained from nine SON neurons.
Within 1 and 3 min after NE bolus injection, EPSC amplitudes
decreased to 46.6 ± 11.0 pA (by 35.6%; P < 0.01) and 44.0 ±
11.4 pA (by 39.1%; P < 0.001), respectively (Fig. 4), whereas
durations decreased to 17.1 ± 4.5 ms (by 50.6%; P < 0.01)
and 12.3 ± 4.1 ms (by 64.5%; P < 0.001). The PPF went from
1.67 ± 0.15 in control to 0.87 ± 0.24 (47.9%; P < 0.01) within
3 min after NE injection (Figs. 5–7). The effects of NE on
EPSCs were reversible, and 95% recovery from inhibition of
EPSC amplitudes and durations was observed 10 min after NE
injection. Intracellular diffusion of GDP-β-S (0.5 mM), H2 (0.5
mM) and BAPTA (10 mM) did not block NE-induced sup-
pression of EPSCs. EPSC amplitudes were 102.6 ± 9.3 pA in
control (n = 20), and decreased to 62.9 ± 8.3 pA (by 38.7%;
P < 0.001) and 45.0 ± 9.7 (by 56.1%; P < 0.001) within 1 and
3 min after NE injection, respectively. Similar attenuations in
EPSC durations and PPF also were observed. In five SON
neurons bathed with a medium containing 20–25 μM
L-NAMe, EPSC amplitudes were reduced from 59.3 ± 10.3
pA in control to 31.4 ± 8.0 pA (by 47.1%; P < 0.001) and
29.9 ± 9.3 pA (by 49.6%; P < 0.001) within 1 and 3 min after
NE injection, respectively (Fig. 8A). EPSC durations decreased
from 44.0 ± 5.9 ms in control to 22.3 ± 8.4 ms (49.3%; P <
0.05), whereas the PPF was 1.83 ± 0.10 in control, but de-
creased to 1.13 ± 0.33 (by 38.3%; P < 0.05) within 3 min after
NE treatment. The combination of perfused L-NAMe with
intracellular diffusion of BAPTA did not abolish the effects of
NE on the EPSCs either (n = 2). These results suggest that NE
suppresses EPSCs in SON neurons through presynaptic mech-
anisms without involving NO production.

DiSCUSSION

Neuroendocrine functions in the CNS are subject to contin-
uous control by extrinsic and intrinsic mechanisms to insure
proper and prompt responses to environmental stimuli (Hatton
1990; Hatton and Li 1998a). In this regard, the SON, the
magnocellular neurons of which synthesize and store either
vasopressin or oxytocin, receive massive neural terminal inputs
from other CNS nuclei (Armstrong 1995; Leng et al. 1999).
Transmitters and/or modulators located at presynaptic termi-

nals in the SON include glutamate, GABA, NE, HA, acetyl-
choline, ATP, oxytocin, vasopressin, angiotensin, opioid and
natriuretic peptides, and interleukins. Many of these substances
not only evoke postsynaptic potentials but also activate mem-
brane metabotropic receptors and influence intracellular bio-
chemical processes (Nicoll et al. 1990). Besides those direct
effects on SON neurons, released substances, i.e., modulators,
can influence functions of other synapses. For example, bath
application of oxytocin, or inhibitors of aminopeptidase, an
enzyme that degrades oxytocin, reduced AMPA currents by
~35%, suggesting that endogenous oxytocin can modulate excita-
tory inputs in the SON (Kombian et al. 1997). Oxytocin
also is known to suppress GABAergic synaptic transmission
through presynaptic mechanisms (Brussaard et al. 1997). Ac-

tivation of GABA_B receptors presynaptically inhibits AMPA
and GABA_A currents (Kabashima et al. 1997; Kombian et al.
1996). In the present study, HA was found to suppress non-
NMDA EPSCs in SON neurons by activating H1 receptors.
The suppression was accompanied by little change in the PPF.
The effects of HA remained after blockade of G-protein or
PKC activation or chelation of intracellular Ca2+ cells where
EPSCs were recorded. EPSC suppression, however, was mim-
icked by an increase in cytosolic cGMP but partially eliminated
by NOS inhibition. When combined with intracellular Ca2+
chelation, NOS inhibition could completely eliminate EPSC
suppression induced by HA. These results support the hypoth-
esis that HA induces NO production in SON cells, and then NO
postsynaptically suppresses the EPSCs in the same and/or
nearby neurons. Bekkers (1993) reported that in isolated or
cultured hippocampal pyramidal neurons, HA enhances
NMDA, but not AMPA, synaptic currents via activating H3
receptors. Other studies also showed that HA enhances NMDA
currents in hippocampal neurons without involvement of any
HA receptor type known (Saybilisi et al. 1995; Vorobjev et
al. 1993). To the best of our knowledge, therefore this is the first
report regarding inhibitory effects of HA on non-NMDA syn-
aptic currents via mediation by H1 receptors.

Constituting more than one-quarter of the nerve terminals on
SON neurons (Meeker et al. 1993; van den Pol et al. 1990),
glutamatergic inputs are critical in the regulation of hypothal-
Released from presynapses, glutamate can bind to postsynaptic
non-NMDA (mainly AMPA) and NMDA receptors to accom-
plish neurotransmission (Mayer and Westbrook 1987; Oza-
w et al. 1998). SON neurons possess both non-NMDA and
NMDA receptors (Meeker et al. 1994a), the activation of
which usually causes membrane depolarization and burst ac-
tivities (Gribkoff and Dudek 1990; Hu and Bourque 1991;
Moos et al. 1997; Wuarin and Dudek 1993; Yang et al. 1995).
In the present study, EPSCs were obtained from SON cells
perfused with a medium containing 3 mM Mg2+ and voltage-
clamped around −70 mV, procedures that separate non-
NMDA currents from NMDA ones (Bekkers and Stevens
1993; Mayer and Westbrook 1987). Current isolation also was
confirmed pharmacologically by application of CNQX and
APV (Young and Fagg 1990), with the former greatly reducing
or eliminating the EPSCs and the latter having little effect.
These results are consistent with the previous reports showing
that in in vitro preparations most excitatory synaptic inputs to
SON neurons are mediated by non-NMDA receptors (Gribkoff and Dudek 1990; Kabashima et al. 1997; Kombian et al. 1997; Wuarin and Dudek 1993).

Being evoked in cells displaying phasic or nonphasic patterns of firing, EPSCs are likely to have been obtained from both vasopressin and oxytocin neurons. There is an increasing body of evidence, however, suggesting that oxytocin and vasopressin neurons are controlled differently by glutamatergic inputs. Immunocytochemical experiments using an antibody against glutamate receptor subunit 3 (GluR3) in the rat hypothalamus reveal that these AMPA receptors are located in the anterodorsal parts of the SON, and 45.8% of oxytocin neurons contain GluR3 immunoreactivity (Ginsberg et al. 1995). In contrast, only 1.1% of vasopressin neurons were found to be GluR3 immunopositive. Spontaneous miniature AMPA currents have been shown to have larger amplitudes and longer durations in oxytocin, than in vasopressin, neurons (Stern et al. 1999). Neuronal activities of putative oxytocin neurons, as well as oxytocin release during lactation are preferentially increased by AMPA receptor activation (Parker and Crowley 1993; Richardson and Wakerley 1997), whereas NMDA receptors are involved in the regulation of rhythmic firing pattern and mediate EPSPs in vasopressin neurons (Hu and Bourque 1992; Moos et al. 1997; Yang et al. 1994). Water deprivation, a strong stimulus for vasopressin secretion due to an increased plasma osmolality and a decreased blood volume, significantly raises NMDA, but not non-NMDA, receptor densities in the SON (Meeker et al. 1994b). Therefore suppression by HA of non-NMDA EPSCs observed in the present experiments might mainly represent one source of inhibition to oxytocin neurons and thus to oxytocin release.

HA, injected into the brain ventricles or SON, is known to elevate plasma vasopressin concentration and induce antidiuresis (Bennett and Pert 1974; Dogterom et al. 1976; Tuomisto and Eriksson 1979; Tuomisto et al. 1984). HAergic projections from the TM, and HA receptors have been located in the SON (Inagaki et al. 1988; Palacios et al. 1981; Weiss et al. 1989). An increase in HA release in the hypothalamus is detected in response to electrical stimulation of the TM (Akins and Bealer 1993), dehydration, or injection of hypertonic saline (Akins and Bealer 1990; Kjaer et al. 1997; Kombian et al. 1997; Kabashima et al. 1997; Kombian et al. 1997; Kjaer et al. 1994). HA predominantly excites putative vasopressin SON neurons, enhances DAPs and promotes phasic firing (Armstrong and Sladek 1985; Haas et al. 1975; Li and Hatton 1996; Smith and Armstrong 1993). In contrast, HA has no effect on, or even hyperpolarizes, the membrane in most oxytocin neurons (Smith and Armstrong 1993). TM stimulation induces fast IPSPs in oxytocin neurons, and these are blocked by H1-antagonists (Yang and Hatton 1994). There is no direct evidence so far suggesting that HA can increase oxytocin secretion either (Hatton 1990; Onodera et al. 1994). Taken together, the present results support the hypothesis that HA-induced suppression of EPSCs functions as a supplementary mechanism to coordinate neuroendocrine activities in the SON. Under conditions of increased demand for vasopressin release, HA reduces the AMPA receptor-mediated glutamatergic drive to oxytocin neurons while exciting vasopressin neurons and promoting phasic firing, in such a way as to preferentially restore, e.g., water balance with little change in other neuroendocrine activities. It is also possible that this mechanism participates in preventing premature oxytocin release before parturition, as suggested in one study (Luckman and Larsen 1997).

Our results imply that HA-induced suppression of EPSCs is mediated by H1 receptor activation. It is unlikely that H2 and H3 receptors are involved because agonists of these receptors failed to mimic the effects of HA. The finding that EPSC suppression was abolished by H1 receptor antagonism also excludes a direct action of HA at AMPA receptors. H2 receptors in the membrane usually are coupled to G proteins, and activation of these receptors can increase the activities of PLC, PKC, and NOS, eventually resulting in NO production and release of Ca2+ (Hatton and Yang 1996; Leurs et al. 1995; Li and Hatton 1996; Li et al. 1999). Because intracellular diffusion of GDP-β-S, H2, and BAPTA did not interrupt EPSC suppression, it is possible that HA’s effects are primarily indirect, i.e., acting at neighboring cells and/or presynaptic terminals first. EPSC suppression could have been a result of local release of neuromodulators after the HA stimulus. A reduction in transmitter release from the presynapses also would cause a similar EPSC suppression. Although many neuromodulators are supposed to be released in response to HA treatment, in the present study we only examined whether NO, a common intercellular modulator (Garthwaite 1991; Zhang and Snyder 1995), plays any role in HA-induced suppression of EPSCs. NO is known to influence synaptic transmission, often through its actions at postsynaptic NMDA receptors and transmitter secretion (Schuman and Madison 1994). In the SON, NOS is abundant (Bredt et al. 1990; Calka and Block 1993; Miyagawa et al. 1994; Sanchez et al. 1994), and NO can increase dye transfer among neurons through gap junctions (Yang and Hatton 1999) and can modulate NMDA currents (Cui et al. 1994). The present results showed that NO inhibition prevented EPSC suppression induced by HA, and cGMP reduced the EPSCs, implying that NO at least partially participates in HA-induced suppression of EPSCs. Our findings are consistent with previous neuroendocrine studies showing that NO controls the release of both vasopressin and oxytocin (Summy-Long et al. 1993; Yasin et al. 1993). The effects of NO donors on EPSCs were not examined here because NO itself can enhance the release of glutamate, dopamine, serotonin, and acetylcholine from the presynapses (Prast et al. 1996; Schuman and Madison 1994).

Further experiments are necessary to identify other potential factors or mechanisms underlying EPSC suppression by HA. Indeed, bath application of L-NAME only partially blocked HA-induced suppression of EPSCs. HA is known to raise [Ca2+]i by increasing Ca2+ influx through membrane channels and Ca2+ release from internal stores (Leurs et al. 1995; Li et al. 1999). Because NOS activity is dependent on [Ca2+], chelation of internal Ca2+ with BAPTA can reduce NO production (Kishi et al. 1996). On the other hand, BAPTA diffusion also can eliminate activation of other intracellular signal pathways due to raised [Ca2+]. These actions of BAPTA might explain why intracellular diffusion of BAPTA, simultaneously with L-NAME treatment, was needed for complete abolition of EPSC suppression by HA.

Noradrenergic inputs play important roles in the regulation of neuroendocrine secretion in the hypothalamus. The SON receives dense noradrenergic innervation from brain stem nuclei and contains a high density of α1 receptors, the activation of which can increase activities of G protein, PLC, and PKC.
and elicits postsynaptic membrane responses in SON neurons (Armstrong 1995; Hatton 1990; Michaloudi et al. 1997; Minneman and Esbenshade 1994). Like HA, NE can depolarize SON neurons, enhance DAPs, and increase neuronal firing rates (Li et al. 1999; Renaud and Bourque 1991; Yamashita et al. 1987). NE also has been demonstrated to suppress spontaneous miniature IPSCs in SON neurons by activating α2 adrenergic receptors in presynaptic terminals (Wang et al. 1998). In the present experiments, NE and HA reduced non-NMDA currents equally. However, several lines of evidence suggest that NE differs from HA in the mechanisms underlying EPSC modulation. First, NE was found to reduce the EPSCs with a remarkable reduction in the PPF, effects that could not be abolished by intracellular treatments of recorded neurons with GDP-β-S, H7, and BAPTA. Second, the maximal responses to NE were observed usually 3–4 min after NE injection, when EPSC suppression by HA already had started to recover. Third, NOS inhibition abolished HA-, but not NE-, induced suppression of EPSCs. These results suggest that NE suppresses non-NMDA currents via a direct action at the presynapses. In contrast, HA attenuation of EPSCs is unlikely to be via presynaptic mechanisms because there were no significant PPF changes after HA treatment. Although both HA and NE are proposed to function as neuromodulators using common intracellular signal transduction pathways (Nicoll et al. 1990), our observations in SON neurons suggest that they can target different sites to bring about a similar effect. As shown in a recent study, both HA and NE enhance the currents underlying DAPs, but only HA’s effects are mediated by Ca2+ release from internal stores (Li et al. 1999).

In conclusion, the present results suggest that HA suppresses non-NMDA synaptic currents in SON neurons through activation of H1 receptors and NOS. It is possible that locally released HA, like oxytocin and GABA (Brussaard et al. 1997; Kabashima et al. 1997; Kombian et al. 1996, 1997), can gate neural signals from other CNS neurons by modulating non-NMDA currents to optimize neuroendocrine activities, particularly during dehydration and the late term of pregnancy. Further studies are needed to probe all intracellular signal transduction pathways involved in mediating the effects of HA and NE on EPSCs.

This work was supported by National Institute on Neurological Disorders and Stroke Research Grants NS-16942 and NS-09140.

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Received 26 August 1999; accepted in final form 13 January 2000.

REFERENCES


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