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NOCICEPTIN/ORPHANIN FQ (N/OFQ) INHIBITS EXCITATORY AND INHIBITORY SYNAPTIC SIGNALING IN THE SUPRACHIASMATIC NUCLEUS (SCN)

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Abstract—Environmental synchronization of the endogenous mammalian circadian rhythm involves glutamatergic and GABAergic neurotransmission within the hypothalamic suprachiasmatic nucleus (SCN). The neuropeptide nociceptin/orphanin FQ (N/OFQ) inhibits light-induced phase shifts, evokes K^+-currents and reduces the intracellular Ca^{2+} concentration in SCN neurons. Since these effects are consistent with a modulatory role for N/OFQ on synaptic transmission in the SCN, we examined the effects of N/OFQ on evoked and spontaneous excitatory and inhibitory currents in the SCN. N/OFQ produced a consistent concentration-dependent inhibition of glutamate-mediated excitatory postsynaptic currents (EPSC) evoked by optic nerve stimulation. N/OFQ did not alter the amplitude of currents induced by application of (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) or *N*-methyl-D-aspartate (NMDA) nor the amplitude of miniature EPSC (mEPSC) consistent with a lack of N/OFQ effect on postsynaptic AMPA or NMDA receptors. N/OFQ significantly reduced the mEPSC frequency. The inhibitory actions of N/OFQ were blocked by ω-conotoxin GVIA, an N-type Ca^{2+}channel antagonist and partially blocked by ω-agatoxin TK, a P/Q type Ca^{2+} channel blocker. These data indicate that N/OFQ reduces evoked EPSC, in part, by inhibiting the activity of N- and P/Q-type Ca^{2+} channels. In addition, N/OFQ produced a consistent reduction in baseline Ca^{2+} levels in presynaptic retinohypothalamic tract terminals. N/OFQ also inhibited evoked $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSC) in a concentration dependent manner. However, N/OFQ had no effect on currents activated by muscimol application or on the amplitude of miniature IPSC (mIPSC) and significantly reduced the mIPSC frequency consistent with an inhibition of GABA release downstream from Ca^{2+} entry. Finally, N/OFQ inhibited the paired-pulse depression observed in SCN GABAergic synapses consistent with a presynaptic mechanism of action. Together these results suggest a widespread modulatory role for N/OFQ on the synaptic transmission in the SCN.

Key words: circadian rhythm, synaptic transmission, excitatory postsynaptic current, inhibitory postsynaptic current, GABA, calcium channel.

The suprachiasmatic nucleus (SCN) of the hypothalamus is the site of an endogenous pacemaker that controls mammalian circadian rhythms. The biological clock does not keep perfect 24-h time, nor are the length of the natural light and dark periods constant through the seasons. Evolution has favored those organisms that have their circadian clock entrained to the environmental light/dark cycle (DeCoursey et al., 1997; Ouyang et al., 1998). The retina is essential to generate phase shifts that entrain the circadian clock to environmental cycles. Light can shift the circadian clock producing phase delays early in the night, phase advances late in the night and no phase change during the day. The anatomical pathway that communicates environmental light information to the circadian clock is the retinohypothalamic tract (RHT), a direct projection from a subset of retinal ganglion cells to the SCN (Moore and Lenn, 1972; Berson et al., 2002). Neurochemically it is glutamate released from RHT terminals acting on both (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors that entrains the circadian clock (Colwell and Menaker, 1992; Ding et al., 1997; Mintz et al., 1999). Afferent pathways from other brain regions have been described that also modulate the light signal to the SCN. For example, it has been observed that 5-hydroxytryptamine (5-HT) can inhibit light-induced phase shifts by activating presynaptic receptors located on RHT terminals (Pickard et al., 1996, 1999; Pickard and Rea, 1997b). Similarly, activation of $GABA_B$ receptors located on RHT terminals will inhibit light-induced phase shifts (Ralph and Menaker, 1989). Activation of either 5-HT or $GABA_B$ receptors reduces the release of neurotransmitter from the presynaptic axon terminals (Jiang et al., 1995, 2000; Pickard et al., 1999).

The neuropeptide nociceptin/orphanin FQ (N/OFQ), when microinjected into the SCN, also inhibits the phase

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Abbreviations: ACSF, artificial cerebrospinal fluid; AMPA, (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; APV, DL-2-amino-5-phosphopentanoic acid; CCD, charge-coupled device; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; CPG 55845, (2*S*)-3-[[(1*S*)-1-(3,4-dichlorophenyl)ethyl] amino-2-hydroxypropyl](phenylmethyl)phosphinic acid; EGTA, ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; EPSCs, excitatory postsynaptic currents; *F*, fluorescence intensity units; IPSC, inhibitory postsynaptic current; mEPSC, miniature excitatory postsynaptic current; mIPSC, miniature inhibitory postsynaptic current; NMDA, *N*-methyl-D-aspartate; N/OFQ, nociceptin/orphanin FQ; OG-5N, Oregon Green 488 BAPTA-5N-AM; ORL1, nociceptin/orphanin FQ receptor; RHT, retinohypothalamic tract; SCN, suprachiasmatic nucleus; UFP-101, [Nphe(1), Arg(14), Lys(15)]N/OFQ-NH(2); 5-HT, 5-hydroxytryptamine.

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advance induced by a light pulse in the late night (Allen et al., 1999). N/OFQ and its receptor (ORL1) are involved in numerous biological functions including pain transmission (Mogil et al., 1996; Heinricher et al., 1997), stress (Jenck et al., 1997; Meis and Pape, 2001), learning and memory (Amano et al., 2000), and locomotor activity (Devine et al., 1996). One commonly observed mechanism of action of N/OFQ in these systems is a reduction of neurotransmitter release via inhibition of voltage-activated Ca^{2+} channels (Knoflach et al., 1996). We therefore hypothesized that the glutamate-mediated synaptic transmission from the retina would be reduced by N/OFQ receptors located on RHT terminals.

GABAergic synapses between SCN neurons are also involved in phase shifting the circadian clock (Smith et al., 1989; Golombek and Ralph, 1994; Tominaga et al., 1994) and bicuculline, a $GABA_A$ antagonist, blocks the light-induced phase delays (Ralph and Menaker, 1989; Gillespie et al., 1997). These data suggest that part of N/OFQ's effect on the light-induced phase shift may involve modulation of GABAergic synaptic transmission. Consequently, we postulated that the release of GABA, the principle neurotransmitter at intra-SCN synapses (Kim and Dudek, 1992; Moore and Speh, 1993; Jiang et al., 1997), might be inhibited by N/OFQ.

By recording evoked and spontaneous synaptic currents in acute brain slice preparations, we show that N/OFQ suppresses glutamate- and GABA-release in a dose-dependent manner during daytime and nighttime recordings. Presynaptic RHT inhibition was shown to involve both a reduction in baseline Ca^{2+} levels and a direct inhibition of voltage-activated N-type and P/Q-type Ca^{2+} channels. These results demonstrate that N/OFQ is an important neuropeptide regulating synaptic neurotransmission in the SCN.

EXPERIMENTAL PROCEDURES

Preparation of brain slices

Male Sprague–Dawley rats (6–8 weeks old) were maintained on a 12-h light/dark schedule for at least 2 weeks before recording. During the light phase, rats were deeply anesthetized with halothane, their brains removed and submerged in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.2, $NaHCO_3$ 20, $MgCl_2$ 4, $CaCl_2$ 0.5, and glucose 11 saturated with 95% O_2/5% CO_2. Coronal (250–300 μm thick) hypothalamic slices containing the SCN were prepared on a vibratome (Leica VT 1000S, Nussloch, Germany), and transferred to the recording chamber in which they were completely submerged in 32 °C ACSF (same as above, except $MgCl_2$ 1.2, $CaCl_2$ 2.4 mM) or 25 °C ACSF for presynaptic Ca^{2+} fluorescence experiments (see below). The Institutional Animal Care and Use Committee of OHSU approved all experimental procedures involving animals and all efforts were made to minimize pain and the numbers of animals used. All studies were in compliance with all US Federal animal welfare requirements.

Patch clamp recording

Experiments were performed with the SCN visualized using infrared differential interference microscopy. A concentric bipolar stimulating electrode (FHC, Bowdoinham, ME, USA) attached to a stimulus isolation unit (Grass Instruments, Quincy, MA, USA) was placed either directly into the SCN (inhibitory postsynaptic current [IPSC] recordings) or into the optic chiasm (excitatory postsynaptic currents [EPSC] recordings). Whole-cell patch clamp recordings were performed 0.5–10 h after slice preparation. Whole-cell patch electrodes had resistances of 5–10 MΩ when filled with a solution containing (in mM): Cs methanesulfonate 87, CsCl 20, $CaCl_2$ 1, HEPES 10, EGTA 11, CsOH 25, ATP 3, GTP 0.3, QX-314 0.5, pH 7.4. Positive pressure was applied as the electrode was advanced onto the surface of a cell and a seal with the cell membrane (resistance: 2–15 GΩ) was obtained by applying negative pressure. The cell membrane was ruptured; currents were measured with an Axopatch-1D amplifier (Axon Instruments, Union City, CA, USA) and sampled at 10 kHz using the data acquisition program PulseFit (HEKA, Lambrecht, Germany) on a Macintosh computer. The holding potential for whole cell voltage clamp recordings was −60 mV, no correction was made for the liquid junction potential.

Synaptic currents were evoked by electrical stimulation (0.2 ms; 10–60 V). Glutamate-mediated currents were pharmacologically isolated by bath application of picrotoxin (50 μM) and their identity confirmed by blocking with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 5 μM) and DL-2-amino-5-phosphopentanoic acid (APV; 50 μM) in selected experiments. $GABA_A$-mediated currents were pharmacologically isolated by bath application of CNQX (5 μM) and APV (50 μM). The IPSC were identified as mediated by $GABA_A$ receptors by their sensitivity to block by picrotoxin (50 μM) and comparing their experimental reversal potential with the calculated Cl^- reversal potential (−47 mV; Jiang et al., 1997; Kim and Dudek, 1992). Picrotoxin, APV, and CNQX were obtained from Sigma (St. Louis, MO, USA).

Application of test compounds

To study the presynaptic modulation of P/Q- and N-type Ca^{2+} channels by N/OFQ receptor activation, ω-agatoxin TK (500 nM), and ω-conotoxin GVIA (1 μM; both Alamone Laboratories, Jerusalem, Israel), were used, respectively. ω-Agatoxin TK or ω-conotoxin GVIA was bath-applied for 20 min through a 100 μm diameter glass pipette placed close to the slice and aimed at the SCN. The ACSF containing the toxins flowed out of the perfusion pipette in the same direction and parallel to the flow of ACSF in the chamber. After the current stabilized during 15 min of toxin application, N/OFQ (1 μM) was added together with the toxin. The effect on current amplitude was measured as the ratio of the EPSC amplitude recorded during toxin application to the control EPSC amplitude. AMPA (100 μM) and NMDA (100 μM) were pressure ejected from a micropipette (2 μm tip diameter) located 30–50 μm from the recorded neuron using a Picospritzer (General Valve, Fairfield, NJ, USA). [Nphe(1), Arg(14), Lys(15)]N/OFQ-NH(2) (UFP-101), an ORL1 antagonist, was purchased from Tocris (Ellisville, MO, USA).

Optical Ca^{2+} measurements

Ca^{2+} sensitive probes were prepared as a 2.5 mM stock of Fura-Red-AM, Fluo-4 AM, Fluo-5F AM, or Oregon Green 488 BAPTA-5N-AM (OG-5N; Molecular Probes, Eugene, OR, USA) dissolved in DMSO, diluted with double strength ACSF (without Mg^{2+}, Ca^{2+} or PO_4^{2-}) to a final concentration of 250 μM and sonicated for 20–30 min. Glass pipettes containing this solution were used to slowly pressure inject (Picospritzer II; General Valve Corporation) the Ca^{2+} probe into the optic chiasm approximately 250–300 μm away from the SCN. The pipette tip was positioned at an angle pointing away from the SCN. A suction pipette adjacent to the site of injection removed extraneous probe. To further reduce the possibility of depositing probe outside of the optic