Cholinergic Modulation of Purinergic and GABAergic Co-Transmission at In Vitro Hypothalamic Synapses

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Received 8 May 2002; accepted in final form 24 July 2002

Jo, Young-Hwan and Lorna W. Role. Cholinergic modulation of purinergic and GABAergic co-transmission at in vitro hypothalamic synapses. J Neurophysiol 88: 2501–2508, 2002; 10.1152/jn.00352.2002. The lateral hypothalamus (LH) is an important center for the integration of autonomic and limbic information and is implicated in the modulation of visceral motor and sensory pathways, including those underlying feeding and arousal behaviors. LH neurons in vitro release both ATP and GABA. The control of ATP and GABA co-transmission in LH may underlie the participation of LH in basic aspects of arousal and reinforcement. LH neurons receive cholinergic input from the pedunculopontine and laterodorsal tegmental nuclei as well as from cholinergic interneurons within the LH per se. This study presents evidence for nicotinic acetylcholine receptor (nAChR)-mediated enhancement of GABAergic, but not of purinergic, transmission despite the co-transmission of ATP and GABA at LH synapses in vitro. Facilitation of GABAergic transmission by nicotine is inhibited by antagonists of (α7)3-containing nAChRs, but is unaffected by an α7-selective antagonist, consistent with a nAChR-mediated enhancement of GABA release mediated by non-α7-containing nAChRs. Activation of muscarinic ACh receptors enhances the release of ATP while concomitantly depressing GABAergic transmission. The independent modulation of ATP/GABAergic transmission may provide a new level of synaptic flexibility in which individual neurons utilize more than one neurotransmitter but retain independent control over their synaptic activity.

INTRODUCTION

The cholinergic systems in the brain have been implicated in a variety of behavioral and cognitive functions, such as working memory and aspects of learning, attention, and arousal. Nicotine alters such cognitive and behavioral functions through specific interactions with nicotinic acetylcholine receptors (nAChRs) found within the diffuse terminals fields of central cholinergic projections (Jones et al. 1999; Levin 1992; Woolf 1991). Nicotine receptors are found in cell bodies, dendrites, and at presynaptic sites in areas that receive cholinergic projections. Recent electrophysiological studies have provided direct evidence for nAChR-mediated synaptic transmission at central synapses (Alkondon et al. 1998; Frazier et al. 1998; Hefft et al. 1999; Jones et al. 1999; Nong et al. 1999). In addition, nAChRs are targeted to synaptic terminals and preterminal domains, consistent with demonstrated effects of ACh and nicotine on the release of a wide variety of neurotransmitters (as reviewed in MacDermott et al. 1999).

Activation of pre- and/or postsynaptic nAChRs of GABAergic neurons has been shown to modulate GABA release in several brain areas (Alkondon et al. 2000; Guo et al. 1998; Len and Changeux 1997; Zhu and Chiappinelli 1999). Our previous study (Jo and Role 2002) and other recent work have demonstrated the release of GABA with other fast-acting neurotransmitters (Jo and Schlichter 1999; Jonas et al. 1998; Keller et al. 2001). At GABA-glycine synapses, the neurotransmitters are packaged within the same vesicles by the vesicular inhibitory amino acid transporter (Gasnier 2000) and the release of both GABA and glycine is modulated by presynaptic GABAB receptors (Jonas et al. 1998). Thus it appears that the contribution of GABA and glycine to the inhibitory control of postsynaptic excitability is coordinately regulated.

The present study tested whether the co-transmission of ATP and GABA in lateral hypothalamic (LH) (Jo and Role 2002) are modulated by cholinergic agonists in general and whether the effects of cholinergic modulators differentially alter purinergic versus GABAergic transmission in particular. The neurons of the lateral hypothalamic area (LHA) are important for behavioral aspects of feeding and arousal (Wille et al. 2001) and comprise an important target of cholinergic neurons from the pedunculopontine and laterodorsal tegmental nuclei (Chennelli et al. 1999) as well as within the LH (Tago et al. 1987). Reciprocal interactions of the LHA with the cholinergic pedunculopontine and laterodorsal tegmental nuclei may underlie the participation of LH in feeding and reinforcement. Several subtypes of both nicotinic and muscarinic AChRs (mAChRs) are expressed within the LH including α4-, α7-, α8-, and β2-type mAChRs and m1- and m2-type mAChRs (Britto et al. 1992; Ehlert and Tran 1990; Okuda et al. 1993; Wei et al. 1994). We tested whether the coordinate release of ATP and GABA was inextricably linked to coordinate modulation of transmission by cholinergic agonists. These studies revealed that cholinergic modulators exert independent control over GABA versus ATP transmission, suggesting potential mechanisms whereby synapses that utilize more than one neurotransmitter nevertheless retain the signaling flexibility afforded by differential, heterosynaptic modulation (MacDermott et al. 1999).

METHODS

Neuronal cultures

Primary cultures of LH neurons were prepared from embryonic day 11 chick using the following protocols (also see Jo and Role 2002).

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The region of the hypothalamus was identified on the ventral aspect of the brain with the exterior border delineated by the optic chiasm and the posterior border delineated by the infundibular stalk. The lateralmost portions of the demarcated area were excised, microdissected, and incubated at 37°C in divalent cation-free Earle’s balanced salt solution (EBSS, Gibco) containing papain (20 U/ml Sigma) and L-cysteine (1 mM, Sigma). After 20 min of incubation the tissue was mechanically dispersed by repeated passage through a heat-polished pasteur pipette. Papain activity was neutralized by adding 10 ml EBSS containing bovine serum albumin (BSA, 1 mg/ml Sigma) and the preparation was centrifuged for 5 min at 300g. The preparation for plating was collected by removing the supernatant and resuspending the pelleted neurons in complete medium composed of DMEM (Gibco), heat-inactivated horse serum (10% v/v, Gibco), chick extract (10% v/v, house-made), penicillin and streptomycin (50 IU/ml for each, Gibco), and NGF (10 nM). LH neuron preparations obtained from approximately 12 E11 chick hypothalami were plated on six 35-mm poly-l-ornithine-coated tissue culture plastic dishes. The culture media were replaced once each week and LH neurons were maintained until use in a water-saturated atmosphere (95% O2-5% CO2) at 37°C.

Electrophysiological recording

Electrophysiological recordings were conducted between 7 and 15 days after plating. Membrane currents were recorded at room temperature (20–22°C) with an Axopatch 200B amplifier (Axon Instruments) in the perforated patch-clamp configuration using amphotericin B. The external solution contained (in mM) 135 NaCl, 5 KCl, 2.5 CaCl2, 1 MgCl2, 5 HEPES, and 10 glucose, pH 7.3. 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 nM; and DL-APV, 50 μM) were continuously present in the external solution. The amphotericin B (Sigma) stock solution (30 μg/ml) was prepared in DMSO just before the recording session. The pipette was first filled at the tip with internal solution containing (in mM) 70 Cs SO4, 9 CsCl, and 10 HEPES, pH 7.3, and then was back-filled with the same solution containing amphotericin B (150 μg/ml). Under these conditions, the equilibrium potential for Cl− ions (ECl) was approximately −70 mV. Adjustment of the equilibrium potential for Cl− ions (ECl) to −70 mV and Ecation, to 0 mV allowed recording of cation-mediated components of synaptic transmission at a holding potential (VH) of −70 mV and Cl− ions-mediated component at a VH of 0 mV.

Eliciting evoked postsynaptic currents (PSCs) and detection of miniature PSCs (mPSCs)

For extracellular stimulations, a double-barreled electrode (World Precision Instruments), filled with extracellular solution, was placed in contact with the cell body of a potential presynaptic partner of the recorded neuron. Stimulation was performed with short single or pairs of stimuli [interval: 300 ms for GABAergic evoked inhibitory postsynaptic currents (eIPSCs) and 150 ms for purinergic evoked excitatory postsynaptic currents (eEPSCs)]. Stimulation with paired and unpaired stimuli revealed that the peak amplitude of mPSCs decayed monotonically with a 2 ms time constant. For each experiment, the threshold for detection was set >5 pA (approximately 2 times the peak-to-peak noise). Recordings were also visually inspected to verify the detection of small amplitude events.

For analysis of the decay phase of mPSCs, the events were selected on the basis of the following criteria: 1) stable baseline recording both before the rise and after the end of mPSC, 2) single events detected with a minimum interval of >100 ms between events, and 3) rise times (10–90% of the peak amplitude of the mPSCs) of acceptable events were <3 ms. For examination of average mPSCs profiles, the events were aligned by their initial rising phase. Voltage and current traces were stored on a videotape recorder and/or the hard drive of the analysis computer after being filtered at 5 kHz by Axopatch 200B. Acquisition and analysis were performed using pClamp6, Axograph 4.0 (Axon Instruments) and Mini analysis 5.0 (Synaptosoft). Student’s t-tests were used to analyze the difference between parameters. The critical value for statistical significance was set at P < 0.05. All statistical results are given as mean ± SE.

Preparation and application of drugs and other reagents

Most reagents were prepared as 1,000× concentrated stock solutions. Bicuculline methiodide, strychnine, TTX, muscarine chloride, and nicotine hydrogen tartrate salt were obtained from Sigma (St. Louis, MO), prepared in distilled water, aliquoted and stored at −20°C. DL-APV (Sigma) was prepared in 100 mM NaOH solution and CNQX (Tocris) in 100% DMSO. The substances to be tested were diluted 1,000× to the final concentrations in extracellular solution immediately prior to the recording session and bath applied at a flow rate of 2–3 ml/min.

RESULTS

Cholinergic modulation by nicotinic AChRs: differential effects on GABA versus ATP transmission

To examine how cholinergic activation might modulate synaptic transmission between LH neurons, we first tested the effects of nicotine on spontaneous (i.e., TTX-resistant) mPSCs mediated by the activation of ATP P2X and GABAA receptors. Nicotine (500 nM) increases the frequency of miniature GABAergic IPSCs (mIPSCs) more than twofold (range 144 to 293%; 212 ± 27% versus untreated control; n = 5 cells; Fig. 1, A and B). Despite such robust increases in GABAergic mIPSCs frequency, neither the amplitude (Fig. 1, C and D) nor the decay kinetics of mIPSCs were affected (control: decay τ = 49 ± 2 ms; mIPSC + nicotine τ = 49 ± 3 ms; n = 5). Such findings are consistent with presynaptic effects of nicotine on GABA release.

The presynaptic modulation of GABAergic transmission is further supported in experiments demonstrating nicotine-induced facilitation of evoked IPSCs (eIPSCs). Brief exposure (1 min) of identified synaptic pairs to nicotine (0.5 μM) induced a reversible facilitation of eIPSCs (Fig. 2, A and C). With paired pulse stimulation, nicotine significantly increased the amplitude of evoked IPSCs, enhancing the first IPSC (I1) more than the second (I2); (Fig. 2, A and B; the percent increase in the amplitude of I1 was 158.6 ± 12% of control; n = 10). Thus nicotine enhanced both the average IPSC and the extent of paired pulse depression (the paired pulse depression ratio is defined as [I1 − I2] × 100/I1; control: 13.9 ± 3.2%; nicotine: 29.7 ± 7.4%; n = 8; P < 0.05; Fig. 2B). The decay time constant of eIPSCs is not significantly affected by nicotine (control: τfast, 20.8 ± 2.5 ms; τslow, 126.7 ± 11 ms; nicotine: τfast, 19.8 ± 2.7 ms; τslow, 119 ± 12 ms; n = 9; P > 0.05). Facilitation of GABAergic transmission by nicotine was inhibited by antagonists of (αβ)α nAChRs, such as mecamylamine (1 μM), but was unaffected by the (αβ)α nAChRs antagonist methyllycyclo-
 nicotine (10 nM; data not shown). The observed effects of nicotine on mIPSCs as well as on evoked GABAergic transmission are consistent with a nicotinic AChR-mediated enhancement of GABA release involving non-α7-containing nAChRs. We next tested for nicotinic AChR-mediated changes in purinergic transmission, expecting that the release of ATP, like GABA, would be enhanced by nicotine. Contrary to our expectation, we found that neither evoked nor spontaneous (TTX-resistant) purinergic transmission was significantly affected by activation of nicotinic AChRs. Application of nicotine (500 nM) at the same synapses where we had observed nicotine-enhanced GABAergic spontaneous transmission revealed no change in the frequency of miniature purinergic EPSCs (Fig. 3, A and B; see also Fig. 1). In five neurons receiving both GABA and ATP input, nicotine (500 nM) increased the frequency of miniature GABAergic IPSCs (mIPSCs) more than twofold, whereas the frequency of ATP P2X receptor-mediated mEPSCs remained equivalent to control (104 ± 9% vs. nicotine versus control). Nicotine changed neither the mean amplitude nor the distribution of amplitudes of the mEPSCs (mean amplitude; control: −11 ± 1 pA; nicotine: −11.3 ± 0.8 pA; n = 5 cells; Fig. 3, C and D). Nicotine was also without effect on the decay time constants of ATP receptor-mediated mEPSCs (decay; control: 15.9 ± 2 ms; nicotine: 16.4 ± 3 ms; n = 5 cells; Fig. 3D).

To test whether nicotine alters evoked transmission medi-
ated via ATP P2X receptors, we examined purinergic excitatory postsynaptic currents evoked by suprathreshold stimulation of presynaptic inputs (eEPSCs) before and after nicotine treatment. The amplitude of purinergic eEPSCs was unaffected by application of nicotine (500 nM). Although nicotine enhanced the frequency of GABAergic mIPSCs in this neuron, it was without effect on purinergic mEPSCs (V_H = −70 mV). A: cumulative probability histograms of inter-event interval before and after the application of nicotine. C and D: histograms showing the distribution and the cumulative probability of amplitude of mEPSCs before and after the application of nicotine. Inset: superimposition of 40 traces of mEPSCs and an average of 40 traces in control and nicotine. E1: representative current traces of purinergic eEPSCs recorded under the conditions in which glutamate, glycine, and GABA_A receptors are blocked by the continuous presence of antagonists. Recordings shown are from before (control), during (nicotine), and after the application (washout) of nicotine. There is no change in the amplitude of evoked purinergic EPSCs (eEPSC; control: −116.6 ± 57.1 pA, nicotine: −124 ± 58.2 pA; P > 0.05; n = 7 cells). V_H = −70 mV. Each trace represents an average of 5 consecutive traces. E2: superimposition of the paired-pulse-evoked EPSCs reveals no difference in the extent of PPD. F: average PPD ratio is shown before and after treatment with nicotine; there is no significant difference (n = 6; P > 0.05). G: plot of evoked purinergic eEPSCs versus time of recording. The amplitudes of the first eEPSC of each neuron were normalized to allow comparison of nicotine effects among the 10 neurons tested. The solid bar indicates the time of nicotine application.

FIG. 3. In contrast to enhancing GABAergic transmission, nicotinic AChR activation has no effect on spontaneous or evoked purinergic transmission. A: recording of purinergic miniature excitatory postsynaptic currents (mEPSCs) from the same neuron shown in Fig. 1A before (control), during (nicotine), and after application (washout) of nicotine (500 nM). Although nicotine enhanced the frequency of GABAergic mIPSCs in this neuron, it was without effect on purinergic mEPSCs (V_H = −70 mV). B: cumulative probability histograms of inter-event interval before and after the application of nicotine. C and D: histograms showing the distribution and the cumulative probability of amplitude of mEPSCs before and after the application of nicotine. Inset: superimposition of 40 traces of mEPSCs and an average of 40 traces in control and nicotine. E1: representative current traces of purinergic eEPSCs recorded under the conditions in which glutamate, glycine, and GABA_A receptors are blocked by the continuous presence of antagonists. Recordings shown are from before (control), during (nicotine), and after the application (washout) of nicotine. There is no change in the amplitude of evoked purinergic EPSCs (eEPSC; control: −116.6 ± 57.1 pA, nicotine: −124 ± 58.2 pA; P > 0.05; n = 7 cells). V_H = −70 mV. Each trace represents an average of 5 consecutive traces. E2: superimposition of the paired-pulse-evoked EPSCs reveals no difference in the extent of PPD. F: average PPD ratio is shown before and after treatment with nicotine; there is no significant difference (n = 6; P > 0.05). G: plot of evoked purinergic eEPSCs versus time of recording. The amplitudes of the first eEPSC of each neuron were normalized to allow comparison of nicotine effects among the 10 neurons tested. The solid bar indicates the time of nicotine application.

Cholinergic modulation of GABA and ATP transmission by muscarinic receptor activation

As endogenous ACh, unlike nicotine, activates muscarinic as well as nicotinic AChRs, we also tested the effects of mACHR activation on GABA and ATP co-transmission in LH.
Such studies revealed that activation of mAChRs at LH synapses modulates both GABAergic and purinergic transmission. In addition, we found that the effects of muscarinic receptor activation contrast with those elicited by nicotine.

Activation of muscarinic receptors depressed both spontaneous and evoked GABA release. The frequency of GABAergic mIPSCs was decreased by 45.4 ± 5% of control levels (Fig. 4, A and B; range 28 to 62; n = 8), although neither the amplitude distributions nor the decay time constants of the GABAergic mIPSCs were altered (Fig. 4, C and D; mean amplitude; control: 12.6 ± 1 pA, muscarine: 11.8 ± 1 pA; n = 8; P > 0.05; decay; control: 53.7 ± 3.3 ms, muscarine: 51.6 ± 2.6 ms; n = 8; P > 0.05). Consistent with the depression of spontaneous GABAergic transmission, the average amplitude of eIPSCs was also decreased (20 ± 2% compared with control) and the PPD ratio was diminished from 27 ± 3% under control conditions to 11 ± 3% by mAChR activation (n = 6; P < 0.05; Fig. 5, A and B). Thus muscarinic AChR activation elicits an overall depression of GABAergic transmission in the LH.

In contrast to the observed depression of GABAergic transmission by mAChR activation, cholinergic activation of muscarinic receptors enhanced purinergic transmission. The frequency of P2X receptor-mediated mEPSCs is increased by the activation of mAChRs. The mean percent enhancement of purinergic eEPSCs was 163 ± 8% of control (n = 5 cells; Fig. 6). Muscarine was without effect on either the amplitude distribution or the decay time constant of purinergic mEPSCs, consistent with an enhancement of ATP release rather than a change in postsynaptic ATP sensitivity (Fig. 6, C and D; mean amplitude, control: −10.9 ± 1.4 pA; muscarine: −11 ± 0.9 pA; control: 12.4 ± 1.5 ms, muscarine: 12.3 ± 1.5 ms; n = 5 cells; P > 0.05). The activation of mAChRs increased the amplitude of stimulus-evoked ATP P2XR-mediated eEPSCs (Fig. 7). The mean percent enhancement of purinergic eEPSCs was 128 ± 17% of control (n = 4 cells). The PPD ratio was also significantly enhanced, increasing from 24 ± 9% in control to 36.5 ± 7% with muscarine treatment (n = 4; P < 0.05; Fig. 7, A2 and B).

The net effects of cholinergic activation on GABAergic and purinergic transmission in the LH are compared and contrasted in Fig. 8. Cholinergic modulation of LH synapses via nicotinic
pathways elicits significant facilitation of GABAergic transmission, without affecting purinergic transmission at ATP/GABA synapses. In contrast, activation of cholinergic pathways via muscarine enhances both spontaneous and evoked purinergic synaptic transmission and depresses GABAergic transmission at the same synapses.

**DISCUSSION**

Our study demonstrates that GABAergic and purinergic transmission in the LH are differently and oppositely modulated by agonists of both ionotropic and metabotropic ACh receptors. Thus, despite the co-transmission by GABAA and ATP P2X-Rs-mediated synapses, purinergic and GABAergic transmission can be modulated independently from one another, depending on the nature of the cholinergic receptor-mediated pathway involved.

Several findings are consistent with a prominent role of presynaptic nicotinic and muscarinic receptors in the modulation of GABA/ATP transmission. First, the observed changes in “mini” frequency were not accompanied by changes in either the amplitude distribution or the decay time constants of spontaneous events mediated by GABAergic or purinergic transmission. Second, the PPD ratio of both GABAergic and purinergic evoked PSCs was significantly affected. In addition, nicotine was without effect on the macroscopic current responses elicited by exogenous application of GABA under the same experimental conditions as the synaptic transmission measurements. Thus the data are consistent with a presynaptic mechanism of cholinergic modulation.

The independent modulation of ATP and GABA transmission in conjunction with the co-transmission of ATP and GABA in LH, as previously demonstrated (Jo and Role 2002), could be explained by several different mechanisms. For example, the positive modulation of GABA release in the absence of a nicotinic effect on purinergic transmission may be due to a low probability of ATP release at ATP-GABA synapses. In this scenario, the activation of nAChRs would not be sufficient to elicit an enhancement of ATP release. To test this possibility, we examined the effect of nicotine on purinergic mEPSCs under the conditions in which both [K+]_ext and [Ca^{2+}]_ext were increased from 5 to 10 and from 2.5 to 5 mM, respectively, to increase the probability of neurotransmitter release. Even under these conditions of elevated purinergic transmission, nicotine was without effect on the frequency of ATP P2XR-mediated mEPSC (data not shown). As such, it seems unlikely that the probability of ATP release per se underlies the differential modulation of GABA versus ATP transmission by nicotine.

Perhaps the simplest model for the differential modulation of ATP and GABA transmission involves the segregation of presynaptic nicotinic and muscarinic receptors combined with functionally separate pools of ATP- versus GABA-containing vesicles. Alternatively there could be the segregation of both pre- and postsynaptic receptors, with storage of ATP and GABA within the same vesicular pool. Both models require
receptors to presynaptic domains. Specification of transmission relies on the targeting of cholinergic agonists per se. The possibility of co-localization of ATP P2X and GABA receptors is raised by our previous studies of the modulation of mPSCs kinetics by flunitrazepam at GABA--ATP co-transmitting synapses (Jo and Role 2002). Such a mechanism, based on the differential detection of transmitters at distinct postsynaptic domains of the same neuron, is akin to models proposing that targeted expression of specific receptor subtypes may underlie the plasticity of converting “silent” to “nonsilent” synapses (Gomperts et al. 1998). It is tempting to speculate that the detection of released GABA versus ATP might be similarly subject to modification, changing with development or with changes in synaptic activity.

Either configuration could account for modulation of ATP/GABA co-transmission in LH. Selective activation of nicotinic pathways associated with “GABA A receptor only” postsynaptic sites would elicit a net enhancement of inhibitory transmission (i.e., GABA > ATP transmission). Activation of cholinergic afferents sufficient to elicit muscarinic receptor-mediated pathways may result in a net disinhibition of synaptic transmission (i.e., ATP > GABA transmission).

Despite the extensive expression of P2X receptors in the hypothalamus, previous work has not emphasized a physiological role for ATP-mediated synaptic transmission in this region, in general, or in the lateral hypothalamus, in particular. The primary physiological role for ATP transmission suggested to date includes aspects of sensory transduction, nociception, thermal hyperalgesia, and mechanical allodynia (Dunn et al. 2000). The alternative model proposing storage of ATP and GABA in distinct synaptic vesicles include prior support for separate storage of ATP versus NE, despite their coordinate release from peripheral sympathetic nerve of guinea pig vas deferens (von Kugelgen and Starke 1991). Other reports consistent with co-transmission, but separate storage of ATP and norepinephrine in peripheral neurons, describe differential effects of prejunctional β-adrenoceptors (Goncalves et al. 1996) and prostaglandin E 2 receptor activation (Trachte et al. 1989). A variation on the former model is raised by recent work of Lester and colleagues (Khakh et al. 2000) showing that the gating of P2X 2- type receptors can inactivate nearby nAChRs. Such inhibitory interactions between presynaptic P2X and nAChRs may underlie the differential modulation of ATP versus GABA transmission by nicotinic agonists per se.

The alternative model proposing storage of ATP and GABA within the same vesicle population of individual LH neurons is clearly quite novel and more complex. Here, independent modulation of transmission relies on the targeting of cholinergic receptors to presynaptic domains specifically apposed either to postsynaptic sites with both GABA and ATP receptors or to postsynaptic sites with GABA receptors, but devoid of ATP receptors. The concept of selective pre- and postsynaptic receptor targeting has been suggested by other work. Previous studies of rat sensory cortical neurons propose that nicotine acts at presynaptic sites, selectively enhancing glutamate release only at sites that include postsynaptic N-methyl-D-aspartate-type glutamate receptors (Aramakis and Metherate 1998).
et al. 2001; Khakh 2001). In contrast, GABA is the primary inhibitory transmitter in the hypothalamus and glutamic acid decarboxylase mRNA is widely expressed within the LH (Elias et al. 2001). Recent studies demonstrate the existence of GABAergic interneuronal synapses in vitro preparations of rat lateral hypothalamus (Gao and van den Pol 2001). Micro-injection of the GABA agonist, muscimol, into the LH depresses food intake (Grandison and Guidotti 1977) and the GABA antagonist, bicuculline, causes an increase in food intake when injected into the LH (reviewed in Bernardis and Bellinger 1996). Several studies support a potentially important role of LH inhibitory circuits in the central control of feeding (for review see Bernardis and Bellinger 1996).

Our prior study (Jo and Role 2002) revealed ATP P2X receptor-mediated transmission in both avian and rodent LH in vitro. In this report, we have extended our analysis of purinergic and GABAergic co-transmission in the LH to assess the potential modulatory role of nicotinic and muscarinic cholinergic agonists. The independent modulation of released ATP and GABA observed at LH synapses in vitro may provide a new level of synaptic flexibility in which individual neurons utilize more than one neurotransmitter but retain independent control over their synaptic activity. Selective facilitation of GABAergic transmission by nicotinic pathways would elicit a net enhancement of inhibitory transmission (i.e., GABA > ATP transmission). Activation of cholinergic afferents sufficient to elicit muscarinic receptor-mediated pathways may result in a net disinhibition of synaptic transmission (i.e., ATP > GABA transmission). The current findings support the notion that cholinergic modulation may exert important control on the net output of LH circuits by selective enhancement of excitatory or inhibitory pathways.

We thank Drs. S. A. Siegelbaum, A. B. MacDermott, and R. Yu for helpful comments on prior versions of this manuscript and T. Davis for technical assistance.

This work was supported by a Grable-Distinguished Investigator Award from National Alliance for Research on Schizophrenia and Depression (NARSAD) and National Institute of Neurological Disorders and Stroke Grant NS-22061 to L. W. Role.

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