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Cholecystokinin Depolarizes Rat Thalamic Reticular Neurons by Suppressing a K^+ Conductance

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SUMMARY AND CONCLUSIONS

1. The thalamic reticular nucleus (nRt) is innervated by cholecystokinin (CCK)-containing neurons and contains CCK binding sites. We used tight-seal, whole cell recording techniques with in vitro rat thalamic slices to investigate the action of CCK on neurons in nRt and ventrobasal thalamus (VB).

2. Brief applications of the CCK agonist cholecystokinin octapeptide (26–33) sulfated (CCK8S) evoked prolonged spike discharges in nRt neurons but had no direct effects on VB neuron activity. This selective excitatory action of CCK8S in nRt resulted from a long-lasting membrane depolarization (2–10 min) associated with an increased input resistance. Voltage-clamp recordings revealed that CCK8S reduced membrane conductance by 0.6–3.8 nS, which amounted to 5–54% of the resting conductance of these neurons.

3. The conductance blocked by CCK8S was linear over the range of −50 to −100 mV and reversed near the potassium equilibrium potential. Modifications of extracellular K^+ concentration altered the reversal potential of the conductance as predicted by the Nernst equation. The K^+ channel blocker Cs^+, applied either intracellularly or combined intra- and extracellularly, blocked the response to CCK8S.

4. The CCK8S-induced depolarization persisted after suppression of synaptic transmission by either tetrodotoxin or a low-Ca^{2+}, high-Mg^{2+} extracellular solution, indicating that the depolarization was primarily due to activation of postsynaptic CCK receptors and not mediated through the release of other neurotransmitters.

5. The selective CCK_A antagonists L364,718 and Cam-1481 attenuated the CCK8S-induced depolarization, whereas the CCK_B antagonist L365,260 had little or no effect on the depolarization.

6. Our findings indicate that CCK8S, acting via CCK_A-type receptors, reduces a K^+ leak current, resulting in a long-lasting membrane depolarization that can presumably modify the firing mode of nRt neurons. Through this effect, CCK actions in nRt may strongly influence thalamocortical function.

INTRODUCTION

Oscillatory rhythms in thalamocortical circuits affect sensory processing and their occurrence is correlated with levels of behavioral arousal (Domich et al. 1986; Hubel 1960; Steriade and Llinás 1988). In addition, abnormal rhythm generation within these circuits may underlie certain pathophysiological states, such as generalized absence epilepsy (Williams 1953). Neuromodulators, including acetylcholine, norepinephrine, and serotonin, can modify these oscillatory rhythms by altering the excitability of neurons in the thalamocortical circuit (McCormick and Prince 1986, 1988; McCormick and Wang 1991; Steriade et al. 1993). Neuropeptides, which are colocalized and presumably coreleased with other neurotransmitters, can also modulate excitability in a variety of CNS neurons (for review, see Bartfai et al. 1988; Kupfermann 1991); however, no information is available concerning neuropeptide actions in the thalamus. Because neuropeptides can induce long-lasting alterations in neuronal excitability at other sites, it is reasonable to hypothesize that neuropeptide actions may be important in the regulation of thalamocortical activities and associated oscillatory rhythms.

A number of neuropeptides, such as cholecystokinin (CCK), vasoactive intestinal peptide, and neuropeptide Y are localized within neurons of the thalamocortical circuit (Allen et al. 1983; Hunt et al. 1987; Kaneko et al. 1985). CCK is present in corticothalamic neurons and thalamocortical relay cells (Burgunder and Young 1990; Ingram et al. 1989; Schiffmann and Vanderhaeghen 1991), both of which innervate the thalamic reticular nucleus (nRt) (Jones 1985). In addition, nRt possesses a high density of CCK binding sites (Pelaprat et al. 1987; Zarbin et al. 1983). Recently, CCK receptor subtypes have been identified and cloned (Wank et al. 1992a,b), and pharmacological identification of CCK actions via these receptor subtypes has become possible in physiological experiments (Chang and Lotti 1986; Lotti and Chang 1989; Woodruff and Hughes 1991). Our previous interests in the intrinsic properties of nRt neurons (Huguenard and Prince 1992), and the potential effects on thalamic rhythms of alterations in nRt activities produced by neurotransmitters (McCormick and Prince 1986) and clinically important pharmacological agents (Huguenard and Prince 1994a,b), led us to examine the actions of CCK in nRt.

In the studies reported here, we used blind slice patch techniques (Blanton et al. 1989) to examine the effects of cholecystokinin octapeptide (26–33) sulfated (CCK8S) on neurons in nRt and the ventrobasal nucleus (VB) of rat thalamic slices. Our results indicate that CCK8S has a long-lasting and selective excitatory action on nRt neurons, produced by decreases in a leak K^+ conductance and mediated via CCK_A receptors. These effects may have an important influence on rhythm generation in intrathalamic circuits (Cox et al. 1994a). Portions of these results have appeared in abstract form (Cox et al. 1994a,b).

METHODS

Young Sprague-Dawly rats (postnatal age 9–16 days) were deeply anesthetized with pentobarbital sodium (55 mg/kg) and decapitated, and the brains were quickly removed and placed into cold, oxygenated slicing medium (~4°C). Tissue slices (400–450 μm) were cut in the horizontal plane using a vibratome (TPI, St. Louis, MO), transferred to a holding chamber containing oxygen-

ated physiological saline at 31°C, and incubated for ≥2 h before recording. Individual slices were then transferred to a submersion-type recording chamber maintained at 35 ± 1°C (mean ± SE) and allowed to equilibrate for ≥30 min before recording. Slices were superfused with oxygenated physiological solution at a rate of 1.3 ml/min. The slicing medium contained (in mM) 2.5 KCl, 1.25 NaH_2PO_4, 10.0 $MgCl_2$, 0.5 $CaCl_2$, 26.0 $NaHCO_3$, 11.0 glucose, and 234.0 sucrose. The physiological saline contained (in mM) 126.0 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2.0 $MgCl_2$, 2.0 $CaCl_2$, 26.0 $NaHCO_3$, and 10.0 glucose. These solutions were gassed with 95% O_2-5% CO_2 to a final pH of 7.4.

Whole cell recordings were made using the "blind" patch technique (Blanton et al. 1989). Recording pipettes (4–6 MΩ when filled with solutions below) were pulled from 1.5 mm OD capillary tubing using a two-stage pipette puller (Narishige PP-83). The intracellular solution used in the majority of recordings contained (in mM) 117 potassium gluconate, 13 KCl, 1.0 $MgCl_2$, 0.07 $CaCl_2$, 0.1 ethylene glycol-O,O′-bis(2-aminoethyl)-N,N,N′,N′-tetraacetic acid, 10.0 N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid), 2.0 Na_2-ATP, and 0.4 sodium guanosine 5′-triphosphate. In some recordings a low-Cl^- (2 mM) intracellular solution was used; results were not different, and data obtained with the two intracellular solutions have been combined. In experiments with intracellular Cs^+, cesium gluconate and CsCl were substituted for potassium gluconate and KCl. The pH of the solution was adjusted to 7.3 using KOH or CsOH, and the osmolality was adjusted to 280 mosM with distilled water (dH_2O). Biocytin (0.5%) was routinely added to the intracellular solution. After formation of a high-resistance seal (1–4 GΩ), brief negative pressure was applied to the pipette and a whole cell configuration obtained. Initial access resistance was 10–40 MΩ and typically increased over the duration of the recording.

An Axoclamp2A amplifier (Axon Instruments, Foster City, CA) was used in bridge mode for voltage recordings or switching single-electrode voltage-clamp mode for current recordings. During voltage-clamp recordings, switching frequencies ranged from 3 to 5 kHz and the headstage was continually monitored to ensure that the current transient had completely decayed before voltage measurements. Voltage-clamp recordings were limited to neurons that had a stable access resistance of <30 MΩ. Voltage and current protocols were generated using PCLAMP software (Axon Instruments) and data were stored on an IBM PC-compatible computer and simultaneously recorded on chart recorder and magnetic tape (Neurodata) for later analysis. In current-clamp recordings (with potassium-gluconate-filled pipettes), apparent input resistance (R_N) was determined from the linear slope of the voltage-current relationship obtained by applying constant current pulses ranging from −110 to 40 pA (1 s). Membrane time constant (τ_m) was estimated by fitting a single-exponential decay function to the membrane response (<10 mV) evoked by a small hyperpolarizing current pulse (20 or 50 pA). During CCK8S application, the change in R_N was determined by the membrane response to single-intensity constant current hyperpolarizing pulses (30–50 pA, 300 ms). Extracellular multiple-unit recordings were obtained using tungsten microelectrodes (Frederick Haer, Brunswick, ME) and a Grass amplifier (bandwidth 0.3–10 kHz), digitized (2 kHz), and stored using Axotape software (Axon Instruments).

The recording pipette was positioned in either nRt or VB, which were easily distinguished with the aid of a dissecting microscope. In addition, neuron types and recording locations were confirmed using intracellular labeling with biocytin. After the recording, the slice was fixed in 4% paraformaldehyde or 4% paraformaldehyde-0.3% glutaraldehyde overnight, and biocytin-filled neurons were processed using previously described techniques (Tseng et al. 1991). Osmium tetroxide (1.0%) in tris(hydroxymethyl)aminomethane buffer was used to enhance the diaminobenzidine reaction product. Slices were also counterstained with cresyl violet to identify the boundaries of VB and nRt.

CCK8S was used in these experiments as a CCK agonist with relatively similar affinities for CCK_A and CCK_B subtype receptors (Woodruff and Hughes 1991). A concentrated stock solution of CCK8S was prepared in 0.1 M NH_4HCO_3 and diluted 300–10,000 times in physiological solution to a final concentration of 0.1–10.0 μM. Concentrated stock solutions of CCK antagonists were prepared in either 1:1 dimethyl sulfoxide:dH_2O (L364,718 and L365,260) or in dH_2O (Cam-1481). All antagonists were diluted to final concentration in physiological solution just before use and bath applied. CCK8S was purchased from Peninsula Laboratories (Belmont, CA) and tetrodotoxin (TTX) from Calbiochem (San Diego, CA). L364,718 and L365,260 were gifts from Merck and Company (Rahway, NJ), and Cam-1481 was a gift from Parke-Davis (Ann Arbor, MI). The remaining chemicals were purchased from Sigma (St. Louis, MO).

CCK8S was applied to the tissue by making short-duration (10–15 s) bolus injections into the input line of the chamber using a motorized syringe pump. This application method was critical for obtaining repeatable responses. In a few initial experiments, bath applications of CCK8S (1–3 μM; 3–6 min) evoked membrane responses that would diminish during prolonged CCK8S perfusion, probably because of receptor desensitization (Boden and Hill 1988). Furthermore, if CCK8S was applied too frequently (bolus injections at intervals of <3 min or bath applications at <10-min intervals), responses became progressively smaller. Brief bolus injections of CCK8S at intervals >6 min elicited consistent responses.

On the basis of the rate of injection of CCK8S into the input line and the chamber perfusion rate, the bath concentration of CCK8S was estimated to be about one fourth of the initial concentration in the injection line, or from 25 nM to 2.5 μM. Control injections of NH_4HCO_3 diluted in physiological saline produced no change in membrane potential or R_N, suggesting that neither the temporary increase in flow rate during the bolus injections nor the low concentrations of NH_4HCO_3 in the CCK solutions affected the recordings.

Results are expressed as means ± SD, except where noted. Regression lines were plotted using the method of least squares. The difference between means was evaluated using the Student's *t*-test for independent samples or, where appropriate, the *t*-test for paired samples. The difference between the means was considered significant when $P < 0.05$.

RESULTS

Excitatory action of CCK on thalamic neurons

The effects of CCK8S were initially tested on the extracellular activity of both VB and nRt neurons. Extracellular multiple-unit recordings revealed that CCK8S (1–3 μM) increased the frequency of spike discharge within nRt (Fig. 1*A*, nRt); however, unit activity in VB was unchanged or only slightly modified (Fig. 1*A*, VB). The increase in extracellular activity could persist for many minutes after a 10- to 15-s application of CCK8S.

Intracellular recordings were obtained to investigate the mechanisms underlying the excitatory action of CCK8S in nRt and to confirm the lack of CCK8S-induced effects in VB. Satisfactory recordings were obtained from 97 nRt neurons and 12 VB neurons with stable resting membrane potentials and overshooting action potentials. The average resting membrane potentials for nRt and VB neurons were −61.4 ± 6.9 mV and −58.7 ± 5.0 mV, respectively, values that were

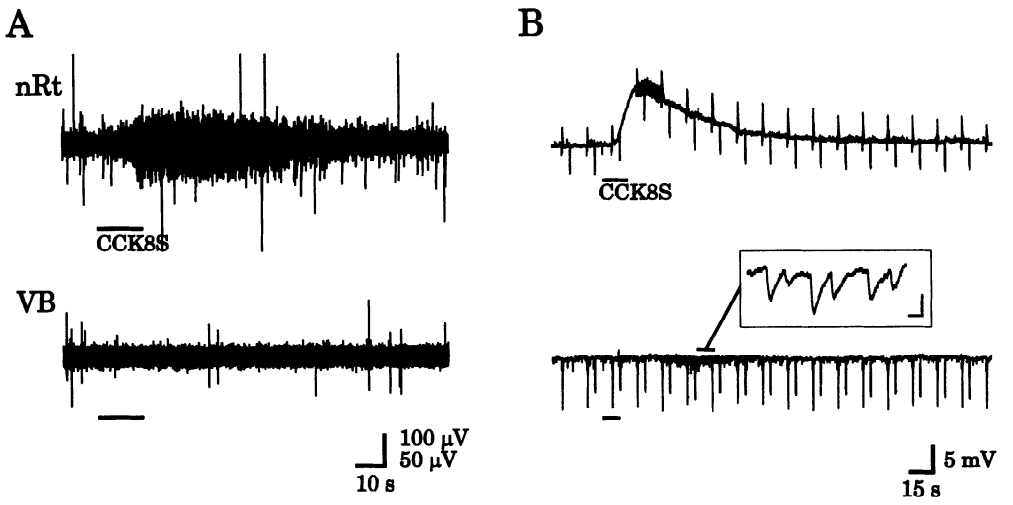

FIG. 1. Excitatory action of cholecystokinin octapeptide (26–33) sulfated (CCK8S) on thalamic reticular nucleus (nRt) neurons. *A*: simultaneous extracellular multiple-unit recordings from nRt and the ventrobasal nucleus (VB). CCK8S (1 μM, 100 μl, applied during bar) elicits an increase in cell discharge in nRt that outlasts the CCK8S application by >1 min. In contrast, there is no detectable change in neuronal activity in VB. *B*: whole cell current-clamp recordings from nRt (*top*) and VB (*bottom*) neurons. CCK8S (3 μM, 100 μl) evokes a depolarization lasting ~2 min in the nRt neuron [membrane potential (V_m) = −62 mV], whereas the same concentration and volume of CCK8S produces no detectable change in the V_m of the VB cell (V_m = −57 mV). The upward deflections in nRt are the synaptic responses evoked by electrical stimulation of internal capsule. The downward deflections in nRt and the larger of the downward deflections in VB are membrane responses to hyperpolarizing current pulses. Note the increased amplitude of the synaptic response during and after the CCK8S-evoked depolarization. The small downward deflections in VB, which are enlarged in the *inset* (calibration: 1 mV, 50 ms), appear to be inhibitory postsynaptic potentials probably due to the release of γ-aminobutyric acid (GABA) from nRt neurons excited by CCK8S.

not significantly different ($P > 0.1$). The nRt neurons had an average R_N of 147.5 ± 58.2 MΩ and a τ_m of 27.1 ± 11.0 ms. The R_N and τ_m of VB neurons were both significantly greater than the values obtained for nRt neurons ($P < 0.05$), averaging 193.9 ± 72.5 MΩ and 44.8 ± 20.7 ms, respectively.

CCK8S evoked a long-lasting depolarization in all nRt neurons tested (Fig. 1*B*, *top*; $n = 90$). The depolarization could exceed spike threshold and result in prolonged discharge (e.g., Figs. 7 and 8). The duration of the depolarizing response ranged from 1 to 8 min after a 10 - to 15-s CCK8S application. As expected from extracellular recordings, CCK8S did not produce consistent changes in the membrane potential or R_N of VB neurons (Fig. 1*B*, *bottom*). However, in some VB neurons, either an increase in the frequency of inhibitory postsynaptic potentials (Fig. 1*B*, *inset*) or a slow, small-amplitude hyperpolarization was observed, coincident with the depolarizations and increased discharge of nRt neurons.

The dose dependence of CCK8S-induced depolarizations in nRt neurons was tested using various concentrations of CCK8S (0.1, 0.5, 1, 3, and 10 μM; Fig. 2*A*) applied by bolus injection. The lowest concentration tested, 100 nM (estimated final bath concentration of 25 nM; see METHODS) evoked a depolarization with an average amplitude of 2.6 ± 0.6 mV and a duration of 139.0 ± 21.9 s in six of eight cells (Fig. 2*A*, 0.1 μM). Increasing CCK8S concentrations produced dose-dependent increases in both the amplitudes and durations of the membrane depolarizations (Fig. 2, *A* and *B*). At higher concentrations, the CCK8S-induced depolarizations could exceed spike threshold (Fig. 2*A*, 3.0 μM). The probability of spike discharge was directly related to the concentration of CCK8S (Fig. 2*C*).

CCK reduces membrane conductance

The CCK-induced depolarization could have been caused by the opening of ion channels whose reversal potential is depolarized from resting membrane potential (Dodd and Kelly 1981; Wu and Wang 1994) or by the closing of channels that have a hyperpolarized reversal potential and are normally open at rest (Boden and Hill 1988; Branchereau et al. 1993). To distinguish between these possibilities, we assessed the change in R_N produced by CCK8S. The depolarization elicited by CCK8S was associated with an increased R_N (e.g., Figs. 1*B* and 2*A*) that persisted when the membrane potential was manually clamped at resting level during the peak of the response (Figs. 2*A* and 6), indicating that the increased R_N was not an indirect result of membrane depolarization. In 38 neurons that were clamped at resting membrane potential during responses to CCK8S, R_N was significantly increased an average of 26 ± 26% compared with control ($P < 0.01$, paired *t*-test). No change in R_N was observed in 6 of the 38 neurons. Although the magnitude of the CCK-induced R_N increase was variable from cell to cell, when more than one concentration of CCK8S was applied to single neurons, the effects were concentration dependent ($n = 7$ of 8). The time course of the resistance change was typically equal to or longer than that of the depolarization (Figs. 2*A* and 6).

To further study the nature of the membrane depolarization produced by CCK8S, we obtained voltage-clamp recordings from nRt neurons. At a holding potential of −50 mV, CCK8S (1–3 μM) evoked an inward current that had an average peak amplitude of 119.4 ± 59.8 pA ($n = 18$; Fig. 3*A*). Slow voltage command ramps (−50 mV to −100 or −110 mV, 2 s in duration, 0.1 Hz) were applied to assess

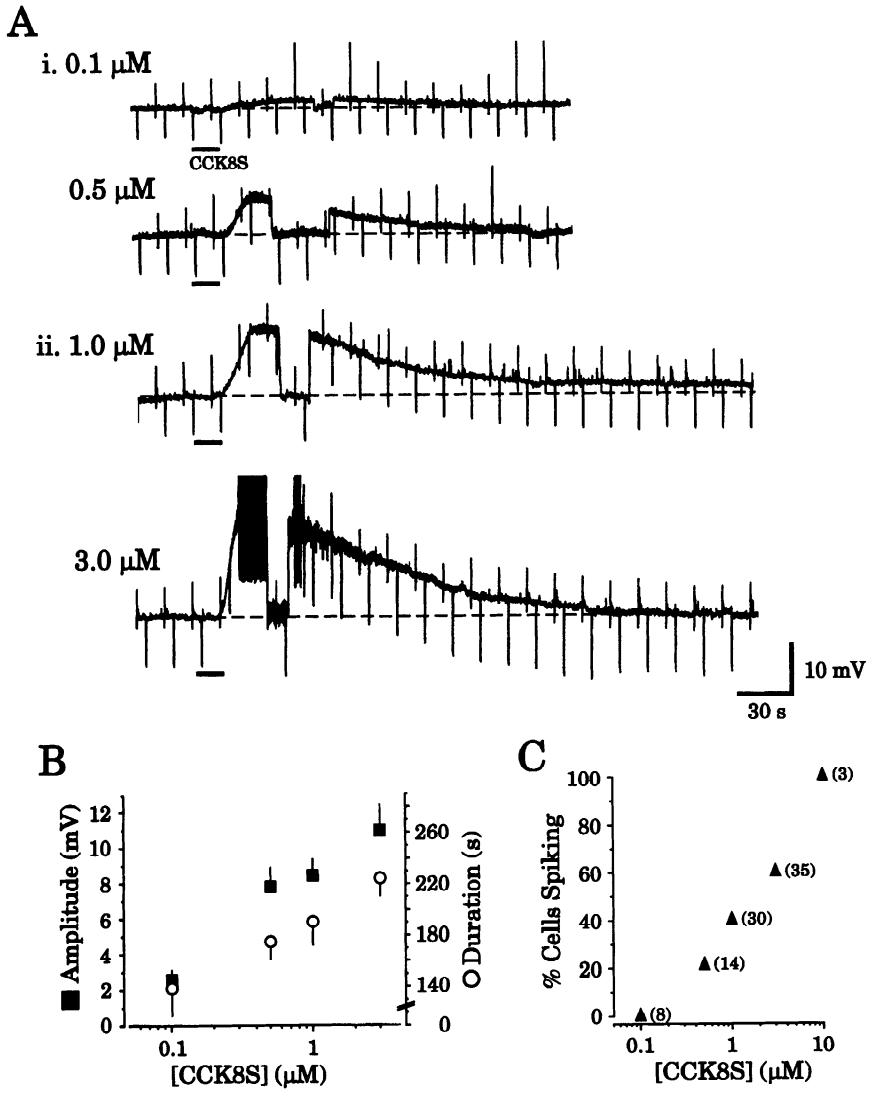

FIG. 2. Concentration-dependent actions of CCK8S on nRt neurons. *A*: dose-dependent responses to CCK8S recorded from 2 nRt neurons. *Ai*: low concentrations of CCK8S (0.1 and 0.5 μM) produce small depolarizations in 1 cell (V_m = −59 mV). *Aii*: recordings from a different nRt neuron (V_m = −66 mV) in which higher concentrations of CCK8S (1.0 and 3.0 μM) produced larger membrane depolarizations. The depolarization resulting from the 3.0-μM CCK8S application exceeded spike threshold (spikes truncated). At peak depolarization in each response, hyperpolarizing current was added to return the membrane potentials to baseline. Negativegoing deflections are the membrane responses to hyperpolarizing current pulses (50 pA, 300 ms, 0.07–0.1 Hz) and positive deflections are synaptic responses evoked by electrical stimulation of internal capsule. Note that the depolarizations are associated with an increased input resistance (R_N) in each neuron. *B*: graph of amplitudes (■) and durations (○) of CCK8S-evoked depolarizations for different concentrations. [CCK8S]: actual concentration delivered into the inflow line before correction for ~4-fold dilution in the superfusate. Amplitude measurements derived only from depolarizations that did not reach spike threshold. Each point represents 6–30 trials. *C*: plot of [CCK8S] vs. the percentage of neurons that reached spike threshold. Numbers in parentheses: numbers of cells tested at each concentration.

the voltage dependence of the CCK8S response (Fig. 3). The CCK8S-evoked inward current was associated with a decreased membrane response to the voltage ramps, indicating a decreased membrane conductance (Fig. 3*A*). Some inward rectification of the membrane response to the voltage ramp was observed at hyperpolarized potentials in control solution (Fig. 3*B*; 8 of 16 neurons). After application of CCK8S, the slope of the membrane responses to the voltage ramps decreased (Fig. 3*B*). The difference in the membrane responses to the ramp voltages in control and CCK8S (I_{diff} in Fig. 3*C*) represents the current suppressed by CCK8S. This current (I_{diff}) was linear over the voltage range tested (Fig. 3*C*) and corresponded to an average conductance change of 1.8 ± 0.9 nS (n = 16).

Strong inward rectification at hyperpolarized potentials (e.g., Fig. 3*B*) confounded measurements of the resting membrane conductance in some neurons. The relatively linear membrane response from a holding potential of −50 to −70 mV was therefore used to calculate the resting conductance, which averaged 9.2 ± 4.2 nS. CCK8S significantly reduced the conductance to 7.4 ± 4.2 nS ($P < 0.01$, paired t-test, n = 16). These data indicate that CCK8S suppresses a voltage-independent resting membrane conductance.

Ionic basis of CCK depolarization

The current responses to voltage ramps (−50 to −100 mV) pre- and post-CCK8S intersected near the potassium reversal potential (Fig. 3*B*), suggesting that CCK8S suppresses a K^+ current. The average reversal potential for this current in control slice solution (2.5 mM KCl), directly measured in 8 of 16 neurons and extrapolated in the remainder, was −114.1 ± 22.3 mV (n = 16; Fig. 3*B*). Perfusion solutions containing various concentrations of K^+ (2.5, 5.0, 7.5, and 10.0 mM) were used to investigate the ionic dependence of the CCK8S-induced responses. Increases in extracellular K^+ concentration ($[K^+]_o$) produced depolarizing shifts in the reversal potential of the current (Fig. 4; n = 5) that were consistent with those predicted for a K^+ conductance from the Nernst equation; however, all values were shifted in the hyperpolarized direction. For example, in Fig. 4*B*, the reversal potentials are −121, −108, and −89 mV for 2.5, 5.0, and 10.0 mM $[K^+]_o$, whereas the predicted values are −105, −86, and −68 mV, respectively. These relatively hyperpolarized reversal potentials could have resulted from inadequate space clamp, because of occurrence of the CCK-induced conductance change at sites distant from

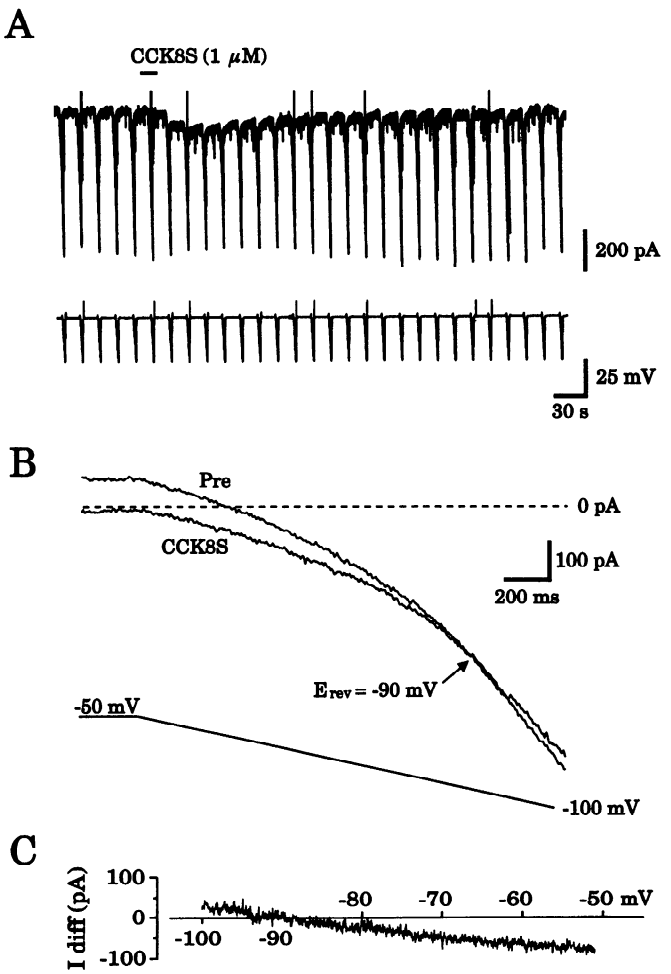

FIG. 3. CCK8S application elicits an inward current associated with a voltage-independent decrease in conductance. *A*: chart record of a voltage-clamped nRt neuron [holding potential (V_{hold}) = −50 mV]. CCK8S (bar, 1 μM, 100 μl) evokes an inward current. The downward deflections are current responses to voltage ramps (−50 to −100 mV, 2 s in duration). Note the decreased amplitude of the current responses (decreased conductance) during the peak of the CCK8S response. *B*: expanded time base of responses to hyperpolarizing voltage ramps before CCK8S application (Pre) and during the peak inward current (CCK8S). Each trace is an average of 4 sequential responses. After CCK8S application there is an inward shift of the holding current at V_{hold} = −50 mV, and a decrease in the slope of the ramp response. The current responses intersect at approximately −90 mV. *C*: CCK8S reduces a voltage-independent conductance that reverses near the potassium equilibrium potential. I_{diff} is the difference between the Pre and CCK8S current responses in *B* plotted vs. voltage. I_{diff}, which is attributable to the actions of CCK8S, has a linear slope <0 (−2.2 nS; r = 0.97), indicating a reduction in conductance. Note that *B* is a current versus time record, whereas *C* is a plot of current vs. voltage. E_{rev}, reversal potential.

the soma. Anatomic reconstructions of nRt neurons indicate that multiple primary dendrites give rise to numerous finer processes (C. Cox, J. Huguenard, I. Parada, and D. Prince, unpublished data; see also Jones 1985) that would presumably be electrotonically distant from the likely site of recording in the soma. Nonetheless, the modifications of reversal potentials by changes in $[K^+]_o$ are entirely consistent with the conclusion that CCK8S suppresses a K^+ conductance.

To further test the involvement of a K^+ conductance in the CCK8S responses, we substituted Cs^+ for K^+ in the recording pipette. The effects of intracellular perfusion with Cs^+ were noted shortly after the whole cell configuration was obtained; the effects consisted of broadened action potentials and decreased resting membrane conductance, presumably due to decreases in K^+ conductance. Under these conditions, in six of eight cells both the inward current and decreased conductance ordinarily produced by CCK8S were blocked (Fig. 5). In the two remaining neurons, intracellular Cs^+ alone did not block the CCK8S actions; however, the addition of CsCl (6 mM) to the bath solution was effective, suggesting incomplete intracellular perfusion with Cs^+. Together with the effects of changes in $[K^+]_o$ noted above, these data clearly indicate that CCK8S reduces a K^+ conductance.

Postsynaptic action of CCK

The reduction of a K^+ conductance by CCK8S may involve direct actions on the postsynaptic nRt neuron or indirect effects due to CCK8S-induced release of other substances from presynaptic neurons or terminals. To rule out possible presynaptic actions of CCK8S, synaptic activity was blocked by adding TTX (1 μM) to the extracellular solution (n = 11). Current-clamp recordings under control conditions revealed the typical CCK8S depolarization that often reached spike threshold with some subthreshold membrane fluctuations (Figs. 1*B*, 2*A*, 6, and 8) and returned to baseline within ~3 min (Fig. 6*Ai, Control*). After TTX-induced suppression of Na^+-dependent action potentials and evoked synaptic responses (Fig. 6*Ai, bottom trace* and *inset*), the CCK8S-induced depolarization persisted in six of six neurons, although the spike discharge was blocked and membrane fluctuations attenuated (Fig. 6*Aii*, TTX). Because the neurons typically generated action potentials during the control CCK8S-induced depolarization, these data were not quantified. The rate of depolarization was not significantly different from control. Whereas the peak CCK8S depolarization appeared unchanged, the late portion of the depolarization was decreased in TTX (e.g., Figs. 6*Ai* and 8). This TTX action may be due to direct reduction of a component of the CCK response or may be effectuated indirectly through attenuation of a postsynaptic voltage-dependent component, such as a persistent Na^+ current. To minimize the contribution of voltage-dependent conductances, the effect of TTX on the CCK8S-induced response was also tested under voltage-clamp conditions (n = 5). Neither peak amplitude nor rate of onset of the CCK8S-induced inward currents were significantly altered in TTX (Fig. 6*Aii;* paired t-test, $P > 0.1$).

In other experiments, a low-Ca^{2+} (0.2 mM), high-Mg^{2+} (6.0 mM) extracellular solution was used to suppress synaptic transmission (Fig. 6*B, bottom trace* and *inset; n* = 7) and further test for pre- versus postsynaptic action of CCK8S. In the majority of these neurons, the control CCK8S-induced depolarization produced little or no spike discharge. In the low-Ca^{2+}, high-Mg^{2+} solution, the CCK8S depolarization was attenuated an average of 15% (Fig. 6*B*). No change in amplitude was observed in two of the seven neurons. Furthermore, the rate of depolarization induced by CCK8S was significantly decreased in the low-Ca^{2+}, high-Mg^{2+} solution (Fig. 6*B;* paired t-test, $P < 0.02$). Although these data further support a postsynaptic action of CCK8S, there

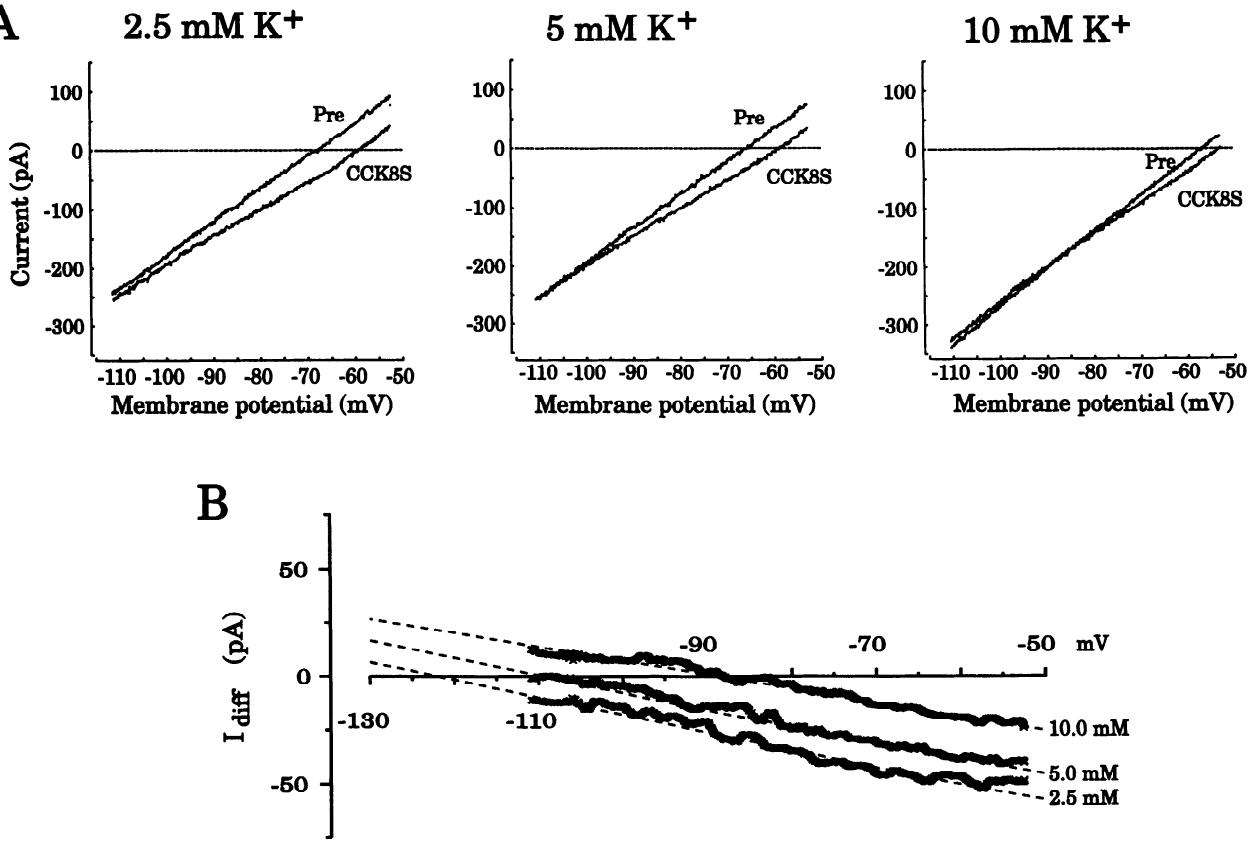

FIG. 4. Effects of extracellular potassium concentration ($[K^+]_o$) on the reversal potential of the CCK8S response. *A*: at each $[K^+]_o$, CCK8S (0.5 μM, 100 μl) elicits an inward current and a reduction in the slope of the membrane responses to command voltage ramps. Increasing $[K^+]_o$ (2.5, 5, and 10 mM) produces a depolarizing shift in the V_m at which the current responses intersect (Pre vs. CCK8S). *B*: reversal potential of I_{diff} (see *A*) decreases with increasing $[K^+]_o$ and is −121 mV (extrapolated), −104 mV, and −87 mV in 2.5, 5, and 10 mM $[K^+]_o$, respectively, in this neuron.

also appears to be involvement of either a Ca^{2+}- or Mg^{2+}-dependent process in generating the CCK8S response.

CCK_A receptors mediate postsynaptic effects of CCK8S

CCK receptors are a heterogenous population, consisting of two primary subtypes, CCK_A and CCK_B (for review, see Woodruff and Hughes 1991). We used the specific CCK_A antagonists L364,718 (Chang and Lotti 1986) and Cam-1481, and the CCK_B antagonist L365,260 (Lotti and Chang 1989), to determine the nature of the receptors underlying the CCK8S-induced membrane depolarization.

CCK_A receptor antagonists attenuated the CCK8S-induced depolarizations in 12 of 12 neurons (Fig. 7). The reduction in depolarization averaged 64 ± 26% in seven neurons with L364,718 (200–500 nM; e.g., Fig. 7*B*) and 68 ± 25% in two cells after perfusion of Cam-1481 (1 μM). Effects on the amplitudes of the depolarization could not be quantified in the remaining three cells because the control CCK8S response contained a prolonged spike discharge; however, in each case the response was clearly attenuated (Fig. 7*A*). The effects of these antagonists were partially reversible in 4 of 12 neurons after a 30- to 90-min wash (Fig. 7, *A* and *B*). We compared the effects of equimolar concentrations of L365,260 and L364,718 on CCK8S-induced depolarizations in four neurons to determine whether a component of the response might be mediated by CCK_B receptors. L365,260 had no effect in three of four cells (Fig. 8, +L365,260) and produced a 25% reduction in the remaining neuron. In contrast, the CCK8S depolarization was attenuated by L364,718 in all neurons (Fig. 8, +L364,718). Overall, the CCK8S response was not significantly different in amplitude or duration from control when slices were exposed to the CCK_B antagonist ($P > 0.1$), whereas the CCK_A antagonist produced a significant attenuation of the CCK8S depolarization ($P < 0.01$), indicating that CCK8S is acting via CCK_A subtype receptors.

DISCUSSION

The thalamus receives a rich peptidergic input from neurons within the brain stem (Lechner et al. 1993) and thalamus (Bendotti et al. 1990; Burgunder and Young 1992), and probably via corticothalamic cells (Burgunder and Young 1990; Schiffmann and Vanderhaeghen 1991). By analogy to results at other sites, peptides can have multiple, prolonged actions mediated by a variety of receptor subtypes coupled to G proteins (e.g., Inoue et al. 1988). Long-term peptidergic effects, such as the reductions in K^+ conductances that occur in neurons of other structures (Boden and Hill 1988; Branchereau et al. 1993; Knoper et al. 1993), would be particularly influential in regulating activities within thalamocortical circuits. The possibility that CCK and other peptides might be released preferentially throughout

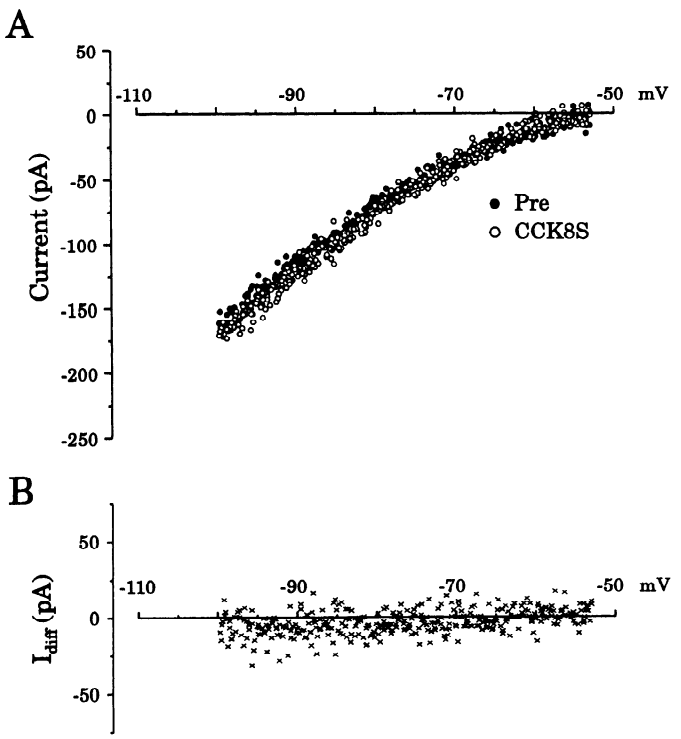

FIG. 5. Intracellular Cs^+ blocks CCK8S actions. *A*: current-vs.-voltage plot obtained from an nRt neuron recorded with Cs^+-filled patch pipette. Responses to voltage command ramps of −53 to −100 mV are shown. Each plot is an average of 4 consecutive responses before (•) and after (○) CCK8S application (1 μM, 100 μl). Note that CCK8S does not elicit an inward current, nor is there a significant decrease in the slope of the response. *B*: subtraction of the 2 responses, Pre and CCK8S, in *A* confirms that Cs^+ blocked the CCK8S action (I_{diff} = 0.2 nS, r = 0.39).

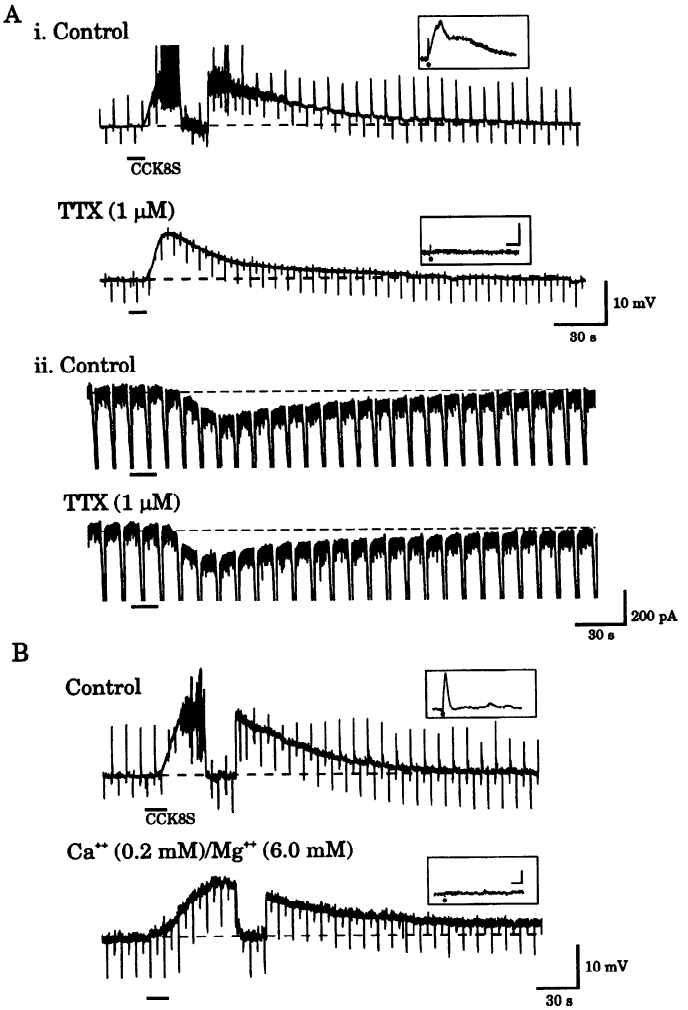

FIG. 6. CCK8S evokes a depolarization in nRt neurons through actions at a postsynaptic site. *Ai*, Control: CCK8S (bar; 3 μM, 100 μl) elicits a depolarization lasting ~2.5 min that exceeds spike threshold at its peak. Positive deflections in *top traces* of *A* and *B* and in Figs. 7 and 8 are excitatory postsynaptic potentials evoked by orthodromic stimulation (*inset;* 0.07–0.1 Hz), and larger negative deflections are responses to hyperpolarizing current pulses. *Ai*, tetrodotoxin (TTX) (1 μM): record obtained 10 min after onset of TTX bath perfusion. TTX suppresses synaptic responses (*inset;* calibration: 5 mV, 100 ms); however, application of CCK8S still elicits a depolarization of similar amplitude and duration, without spike discharge. V_m = −56 mV. *Aii*, Control: chart record of a voltage-clamped neuron (V_{hold} =-50 mV). CCK8S (3 μM, 100 μl) evokes an inward current. The downward deflections are responses to voltage ramps (see Fig. 3), which have been truncated in these records. *Aii*, TTX (1 μM): reapplication of CCK8S during bath application of TTX induced a similar inward current. *B*, Control: CCK8S (3 μM, 100 μl) evokes a similar depolarization and superimposed spike discharge in another neuron. *B*, Ca^{2+} (0.2 mM)/Mg^{2+} (6.0 mM): bath application of low-Ca^{2+}, high-Mg^{2+} solution for 20 min has blocked synaptic transmission (cf. *top* and *bottom insets;* calibration: 5 mV, 100 ms). Application of CCK8S results in a depolarization that has a slower time course and smaller peak amplitude than control response. V_m = −63 mV. Spikes truncated in *top traces* of *A* and *B*.

periods of high-frequency discharge during waking (Steriade et al. 1986) or during the spike bursts that characterize the activities of nRt and relay neurons during normal and pathophysiological rhythm generation (Mulle et al. 1986; Pelaprat et al. 1987) makes it important to assess peptidergic actions in the thalamus.

The results of these experiments provide evidence for functional postsynaptic CCK receptors in the thalamus, as well as details regarding the mechanism underlying actions of this peptide on nRt neurons. CCK8S evokes a robust and prolonged dose-dependent membrane depolarization associated with an increased R_N in neurons of nRt but not VB. This effect is produced by suppression of a voltage-independent potassium leak (K_{leak}) conductance and is mediated predominantly by activation of postsynaptic CCK_A-subtype receptors.

Multiple actions of CCK

CCK produces both excitatory and inhibitory effects in the CNS through direct postsynaptic actions (Boden and Hill 1988; Branchereau et al. 1993; Dodd and Kelly 1981; Jarvis et al. 1992; Wu and Wang 1994), alterations in release of transmitters (Marshall et al. 1991; Sheehan and De Belleroche 1983; Voigt et al. 1986), and modulation of synaptic activity (Branchereau et al. 1992; Delfs and Dichter 1985). The ionic mechanisms underlying direct actions of CCK on membrane potentials differ in various types of neurons. CCK can produce membrane depolarizations associated with a decreased R_N that likely involve an increase in a mixed cation conductance (Dodd and Kelly 1981; Jarvis et al. 1992; Wu and Wang 1994). By contrast, in some neurons CCK-elicited depolarizations are associated with an increased R_N resulting from decreases in a resting K^+ conductance (Boden and Hill 1988; Branchereau et al. 1993; Knoper et al. 1993), an action similar to that in nRt where

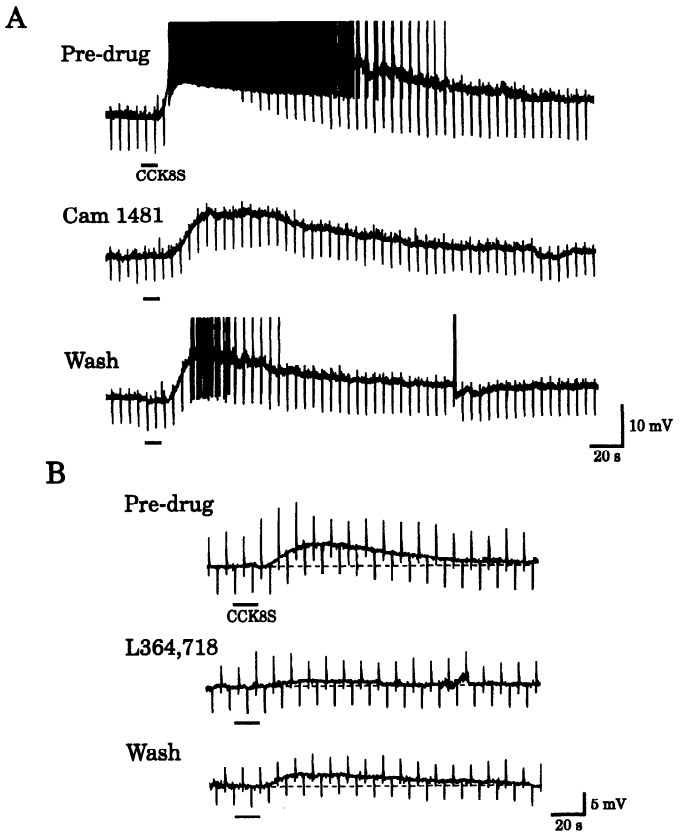

FIG. 7. Cholecystokinin-A (CCK_A) antagonists reversibly attenuate CCK8S-evoked depolarization. *A*, Pre-drug: CCK8S (10 μM) elicits a depolarization with prolonged spike discharge. The response illustrated is the 3rd of 3 consecutive control responses obtained over a period of 30 min. All of the control responses were similar in amplitude and duration, indicating that the responses were stable. *A*, Cam 1481: After a 15-min bath application of the selective CCK_A antagonist Cam-1481 (1 μM), the response to CCK8S is attenuated and does not reach spike threshold. *A*, Wash: CCK8S response partially recovers after 15 min in drug-free solution. $V_m = -62$ mV. *B*, Pre-drug: control response to application of CCK8S (1 μM, 100 μl). *B*, L364,718: bath application of the CCK_A antagonist L364,718 (200 nM, 16 min) results in an ~80% attenuation of subsequent response to the same dose of CCK8S. *B*, Wash: partial recovery of CCK8S response after 45 min in control solution. $V_m = -52$ mV.

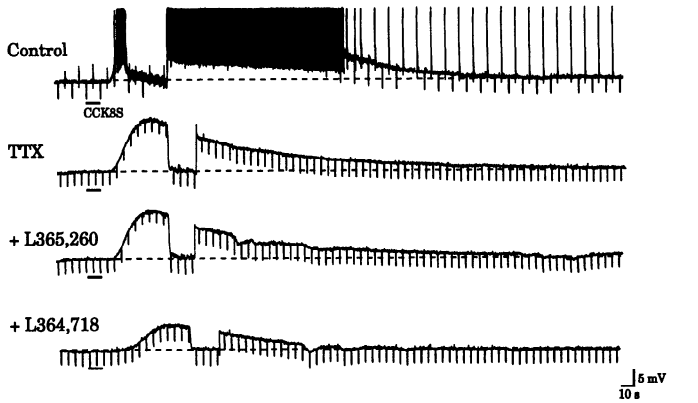

FIG. 8. CCK depolarization is differentially attenuated by selective CCK receptor antagonists. Control: CCK8S (1 μM, 100 μl) elicits prolonged depolarization with superimposed spike discharge. Upward deflections before and after CCK8S depolarization: orthodromic stimuli evoked excitatory postsynaptic potentials before drug application and excitatory postsynaptic potentials followed by spikes after drug application. TTX: after suppression of Na^+-dependent action potentials and synaptic transmission by TTX (1 μM, 10 min), the same dose of CCK8S evokes a robust depolarization with a duration similar to control. +L365,260: addition to the TTX solution of the selective CCK_B antagonist L365,260 (200 nM, 10-min bath application) fails to alter the amplitude or duration of the response to CCK8S. +L364,718: addition of the selective CCK_A antagonist L364,718 (200 nM, 11-min bath application) to the TTX solution results in a 50% attenuation of the CCK8S depolarization. $V_m = -59$ mV.

CCK suppresses a voltage-independent K_{leak} conductance present at resting membrane potential.

CCK binding sites within the CNS are quite abundant and consist of two major receptor subtypes, CCK_A and CCK_B, that have been pharmacologically identified and cloned (Chang and Lotti 1986; Innis and Snyder 1980; Lotti and Chang 1989; Wank et al. 1992a,b; Woodruff and Hughes 1991). A significant density of binding sites is present in nRt, and pharmacological studies using the relatively specific CCK_B agonists pentagastrin and the tetrapeptide CCK4 have suggested that these binding sites may be CCK_B receptors (Gaudreau et al. 1983). Within the CNS, CCK-induced depolarizations and excitatory actions are typically associated with CCK_B receptors (Boden and Hill 1988; Branchereau et al. 1993; Jarvis et al. 1992). However, our data indicate that the CCK8S-induced depolarization of nRt neurons is due to activation of CCK_A and not CCK_B receptors. This is the first described CCK_A-mediated suppression of K^+ conductance in the CNS, although similar results have been observed in sympathetic ganglia (Knoper et al. 1993). Recently, a CCK_A-mediated depolarization, involving an increased mixed cation conductance, has been reported in substantia nigra (Wu and Wang 1994).

Collectively, these data suggest that a given CCK receptor subtype may be associated with multiple effector mechanisms, and further that different CCK receptor subtypes may share common effector mechanisms. In addition to the variety of possible combinations of receptor-effector mechanisms, multiple active forms of endogenous CCK, such as CCK8S and CCK4, have been identified in the CNS (Marley et al. 1984). These endogenous CCK agonists differ in their affinity for the CCK receptor subtypes; for example, CCK4 has a higher affinity for CCK_B receptors than for CCK_A receptors (Woodruff and Hughes 1991). The differential distribution of these various active forms of the peptide and of the multiple CCK receptor subtypes could produce a functional diversity of endogenous CCK actions in the CNS.

After blockade of synaptic transmission with TTX, the CCK8S-induced depolarization persisted, indicating that the response was primarily due to a direct action of the peptide on nRt neurons. However, in low-Ca^{2+}, high-Mg^{2+} solution, the amplitude and the rate of depolarization were both decreased, actions not observed in TTX (cf. Fig. 6, *A* and *B*). These differences could indicate that CCK also acts on presynaptic terminals to release another transmitter, which participated in the postsynaptic response, in an Na^+-channel-independent fashion. A blockade of this CCK-induced release by the low-Ca^{2+}, high-Mg^{2+} solution would thus alter the postsynaptic CCK8S response. CCK has been found to increase dopamine release in basal ganglia (Hökfelt et al. 1986) and to elevate excitatory amino acid levels in rat hippocampus (Migaud et al. 1994). A presynaptic action on cortical neurons to facilitate excitatory synaptic potentials

has also been suggested (Delfs and Dichter 1985). Alternatively, a component of the CCK8S depolarization may be a Ca^{2+}-or Mg^{2+}-dependent postsynaptic action that is unaffected by TTX.

Iontophoretic application of CCK can produce both excitatory and inhibitory actions on extracellular unit activity in rat lateral geniculate nucleus (LGN) (Albrecht et al. 1994). Our data indicate that CCK has excitatory actions in nRt and not in the relay nucleus, VB; thus these findings suggest a difference in CCK effects in VB versus LGN. Anatomically, rodent VB possesses a relatively homogeneous population of relay neurons with virtually no GABAergic interneurons, whereas LGN contains ~10–15% inhibitory interneurons (Ottersen and Storm-Mathisen 1984). CCK binding sites have been localized in both LGN and nRt (Gaudreau et al. 1983; Pelaprat et al. 1987; Zarbin et al. 1983). The mixed effects of CCK in LGN could therefore result from an excitatory action on inhibitory interneurons or diffusion of the iontophoretically applied CCK onto nearby nRt neurons, resulting in inhibition of LGN relay cells. In higher-order mammals, VB also contains inhibitory interneurons that provide a major source of inhibition, in addition to that supplied by nRt. To assess the effects of CCK on intrathalamic circuit behavior, it will be important to determine whether there are excitatory CCK actions on intrathalamic interneurons similar to those in nRt.

Functional significance of CCK actions in nRt

Thalamic neurons generate action potentials in "burst" and "single-spike" firing modes. These patterns of discharge vary with the state of arousal, and they are hypothesized to influence the relay of sensory information from the periphery through the thalamus to the cortex (for review, see Steriade and Llinás 1988; Steriade et al. 1993). The occurrence of burst generation or single spiking, and the transition between these two states, is in part a result of the voltage-dependent intrinsic membrane properties of these neurons (Bal and McCormick 1993; Deschênes et al. 1982; Huguenard and Prince 1994b; Jahnsen and Llinás 1984). Neuromodulators, such as acetylcholine, norepinephrine, and serotonin can alter the firing patterns of thalamic neurons by modifying their membrane potentials (McCormick and Pape 1990; McCormick and Prince 1987, 1988; McCormick and Wang 1991). Stimulation of the brain stem ascending cholinergic system can disrupt oscillatory activities and desynchronize the electroencephalogram, presumably by modifying the firing mode of thalamic neurons (Curró Dossi et al. 1991; Hu et al. 1989; Moruzzi and Magoun 1949). The ionic mechanisms by which these neuromodulators exert their influence involve increases or decreases in K^+ conductances (McCormick and Prince 1987, 1988; McCormick and Wang 1991). Our data indicate that CCK suppresses a K_{leak} conductance in nRt in a manner similar to that underlying the actions of norepinephrine and serotonin (McCormick and Wang 1991); the depolarization evoked by these agents alters the firing mode of nRt neurons through effects on the transient Ca^{2+} current. Thus these multiple "modulators" may converge on common effector mechanisms to produce similar actions in the thalamus.

The reciprocal synaptic relationship between nRt (and the analogous perigeniculate nucleus) and thalamic relay nuclei forms the basis of an intrathalamic neuronal circuit capable of producing certain oscillatory rhythms that have important roles in generation of sleep spindles and pathophysiological activity such as the spike and wave discharges associated with absence-type epilepsy (Huguenard and Prince 1994b; Steriade and Llinás 1988; Steriade et al. 1993; von Krosigk et al. 1993). CCK can disrupt intrathalamic oscillations by depolarizing nRt neurons out of the membrane potential range in which burst firing occurs (unpublished observations). However, the function of CCK may be quite different from that of other modulators in this circuit, which have similar actions when applied to nRt cells (e.g., McCormick and Wang 1991). CCK is found in both thalamocortical and corticothalamic cells that synapse on nRt neurons, in contrast to other modulators that are contained in neurons extrinsic to the thalamocortical circuit and nRt. Thus CCK may serve as an endogenous modulator whose release will depend on patterns or levels of activity in corticothalamic and thalamocortical cells. For example, CCK actions might be most prominent during oscillatory burst discharges of relay neurons, since high-frequency spiking is optimal for peptide release (Bartfai et al. 1988). With successive spike bursts in relay neurons, a slow increase in CCK release would occur in nRt, leading to a slow depolarization that could shift the nRt neurons from burst to single-spike mode. In this way, CCK might serve as an endogenous brake for intrathalamic oscillations.

The dampening of oscillations might be balanced by CCK-induced enhancement of excitatory postsynaptic potentials (EPSPs) in nRt (e.g., Figs. 1*B* and 6) because of the increase in R_N or a presynaptic action. If the CCK-induced increase in R_N is located distant from the soma, as suggested by the hyperpolarized reversal potential, then this postsynaptic modification could have a robust facilitatory effect on nearby dendritic EPSPs. In addition, computer simulations indicate that realistic prolonged Ca^{2+}-dependent bursts in nRt only occur when low-threshold Ca^{2+} channels are predominantly located in the dendritic region of nRt neurons (A. Destexhe, personal communication). We speculate that augmentation of dendritic R_N by low concentrations of CCK that results in little or no depolarization of the soma would enhance EPSPs and increase the probability of evoking a low-threshold Ca^{2+} spike, facilitating burst firing in these neurons. The resulting increase in GABAergic output from nRt onto relay neurons would enhance intrathalamic oscillations (e.g., Huguenard and Prince 1994a). In contrast, increasing CCK concentrations would produce a stronger depolarization, inactivating the low-threshold Ca^{2+} spike and shifting the neuron into single-spike mode. Thus the net effect of CCK on the oscillatory activity would then result from a balance between the relative degrees of membrane depolarization and synaptic enhancement. The differences in the time course of CCK effects on membrane potential and R_N (Figs. 2*A* and 6) indicate that the enhancement of the EPSP may occur when no depolarization is detected by the presumably somatic patch pipette (e.g., Figs. 1*B*, 6*A*, and 8). Thus we hypothesize that CCK may facilitate intrathalamic oscillations by enhancing synaptic responses at lower concentrations, whereas the depolarizing action might predominate at

higher concentrations, resulting in a dampening of intrathalamic oscillatory activity.

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