Low-Threshold Transient Calcium Current in Rat Hippocampal Lacunosum-Moleculare Interneurons: Kinetics and Modulation by Neurotransmitters

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Interneurons from the CA1 lacunosum-moleculare (L-M) region were isolated by trypsin-hyaluronidase treatment and mechanical trituration of the L-M. Interneurons isolated in this manner were multipolar with several dendritic processes and could be distinguished from CA1 pyramidal neurons. The properties of a low-threshold transient (LTT) Ca2+ current were investigated using whole-cell voltage-clamp techniques. The activation threshold of the LTT Ca2+ current was -60 mV, and the peak current, 100 ± 9 pA (mean ± SEM; n = 15), was observed at -30 mV. Ca2+ was the predominant charge carrier because the current was not affected by tetrodotoxin and was abolished in Ca2+-free external solution. Steady state inactivation was observed when the holding potential was positive to -100 mV, and the current was half-inactivated at -84 mV. Complete inactivation occurred at a holding potential of -60 mV. The time-to-peak of the current was highly voltage dependent and ranged from 10 msec at -60 mV to 4 msec at 0 mV. The time constant of inactivation was also voltage dependent and ranged from 27 msec at -60 mV to 12 msec at >-30 mV. Recovery from inactivation to 90% of maximum current occurred within 200 msec.

L-M interneurons receive synaptic inputs from the septum that release ACh or GABA and from the raphe nuclei that release 5-HT. Carbachol, a nonhydrolyzable cholinergic agonist, and 5-HT quickly and reversibly increased the amplitude of the LTT Ca2+ current. Carbachol's actions were blocked by atropine, indicating that this effect was mediated by muscarinic receptors. The actions of 5-HT were blocked by spiperone, a serotoninergic antagonist. Baclofen, which activates GABA_B receptors, reversibly depressed LTT Ca2+ currents. Cholinergic inputs from the septum and serotonergic inputs from the raphe nuclei are both believed to play a role in generating theta rhythm (4-10 Hz) in the hippocampal EEG. LTT Ca2+ currents have been shown to induce rhythmic bursting at theta frequencies in other cell types. This work suggests that muscarinic and serotoninergic enhancement of LTT Ca2+ currents in L-M interneurons could be responsible for generating the theta rhythm in the hippocampus. GABAergic inputs may play a role in terminating the bursting.

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Low-threshold Ca2+ spikes have been observed in vertebrate neurons from several brain regions, including the inferior olive (Llinas and Yarom, 1981), thalamus (Jahnsen and Llinas, 1984a,b), medial septum (Alvarez De Toledo and Lopez-Barneo, 1988), neocortex (Friedman and Gutnick, 1987), spinal cord (Murase and Randic, 1983), deep cerebellar nuclei (Llinas and Muhlethaler, 1988), pars intermedia (Williams et al., 1990), and lateral habenula (Wilcox et al., 1988). A low-threshold transient (LTT) Ca2+ current, first described in chick and rat dorsal root ganglion (Carbonc and Lux, 1984a,b), has been shown to be distinct from the high-threshold Ca2+ currents and has been termed t-type (Nowycky et al., 1985; Fox et al., 1987a,b). Voltage-clamp analysis of acutely dissociated thalamic and lateral geniculate neurons has also indicated that the LTT Ca2+ current underlies the low-threshold Ca2+ spike (Coulter et al., 1989; Hernandez-Cruz and Pape, 1989; Suzuki and Rogawski, 1989). The presence of low-threshold Ca2+ spikes imparts the possibility of rhythmic burst discharge patterns in neurons (Llinas, 1988). In the thalamus, hyperpolarizing inputs deinactivate the low-threshold Ca2+ spike and allow oscillations of membrane potential that occur at approximately 10 Hz (Deschenes et al., 1984; Jahnsen and Llinas, 1984b; Llinas, 1988). Rhythmic bursting at approximately 10 Hz due to low-threshold Ca2+ spikes has also been observed in lateral habenula neurons (Wilcox et al., 1988). Propagation of low-threshold Ca2+ spikes between electrotonically coupled cells could underlie the synchronous rhythmic oscillations of neurons in the inferior olive (Llinas and Yarom, 1986).

Whole-cell voltage-clamp recordings from cultured (Ozawa et al., 1989) and immature hippocampal neurons (Yaari et al., 1987) have revealed LTT Ca2+ currents. Recordings from isolated pyramidal neurons have generally not revealed LTT Ca2+ currents (Kay and Wong, 1987), although a subpopulation of pyramidal neurons may express this channel (Takahashi et al., 1989; Fisher et al., 1990). Intracellular recordings from interneurons in the lacunosum-moleculare (L-M) area of the CA1 region suggested that these cells may have low-threshold Ca2+ spikes (Lacaille and Schwartzkroin, 1988a,b). Burst discharges were only observed when cells were hyperpolarized, a characteristic indicative of low-threshold Ca2+ spikes (e.g., Jahnsen and Llinas, 1984a,b). In contrast, basket cell interneurons of the stratum pyramidale and interneurons in the strata oriens–alveus do not have any indication of low-threshold Ca2+ spikes (Lacaille et al., 1989; Lacaille and Williams, 1990).

The presence of low-threshold Ca2+ spikes in L-M interneurons may be important in explaining the generation of rhythmic activity such as theta, which can be induced by muscarinic stimulation in the isolated hippocampal slice (MacVicar and...
Tse, 1989; Tse and MacVicar, 1989). Theta rhythm is a naturally occurring rhythm, (4-10 Hz) of the hippocampal EEG that can be observed in the conscious animal (Bland, 1986). This rhythm is associated with the bursting frequency observed in cells with prominent low-threshold Ca²⁺ spikes. Synaptic inputs from the septum and serotonergic inputs from the raphe nuclei, which are believed to play a role in the generation of theta rhythm, have been shown to synapse on L-M interneurons (Freund and Antal, 1988; Freund et al., 1990). It has been suggested that phasic activity in septal cholinergic inputs could rhythmically drive hippocampal interneurons, which would then rhythmically inhibit pyramidal neurons (Stewart and Fox, 1990). We have investigated the possibility that these synaptically released transmitters enhance LTT Ca²⁺ currents in L-M interneurons, which could modulate endogenous bursting activity in these cells. To promote this study, we have developed a method for acutely isolating L-M interneurons for two reasons: (1) whole-cell voltage clamp of acutely isolated cells facilitates the rigorous isolation and identification of an ion current, and (2) the sparse distribution of L-M interneurons means that it is very difficult to obtain intracellular recordings reliably from these cells in the in vitro brain slice. In the present article, results from whole-cell voltage-clamp experiments identify and describe the kinetic properties of LTT Ca²⁺ currents in L-M interneurons. We also demonstrate enhancement of LTT Ca²⁺ currents by muscarinic and serotoninergic activation and depression by GABAergic activation.

Some of this work has been presented in abstract form (Fraser and MacVicar, 1990).

Materials and Methods

Acute dissociation. Experiments were performed on hippocampal interneurons acutely isolated from the L-M utilizing a revised method of Kay and Wong (1986). Hippocampal slices (500 µm) were obtained from male Sprague-Dawley rats (postnatal, 3-4 weeks old). The slices were incubated for 1 hr in artificial cerebrospinal fluid (aCSF) containing (in mM) 124 NaCl, 5 KCl, 1.3 MgCl₂, 2 CaCl₂, 26 NaHCO₃, and 10 d-glucose (pH 7.35; 280 mOsm). Slices were then transferred to a spinner flask (Belco Glass Corp.) with a magnetic stirrer filled with 30 ml of low-Ca⁺ aCSF at 35°C containing (in mM) 124 NaCl, 5 KCl, 3.2 MgCl₂, 1 CaCl₂, 26 NaHCO₃, 10 d-glucose; (pH 7.35; 280 mM), to which 1 mM kynurenic acid, 40 mg of tetrodizine (9000 U/mg), and 20 mg of hyaluronidase (1500 U/mg) were added. The slices were placed at a rate sufficient to buoy them up and 99% O₂/1% CO₂ was introduced continuously. After 90 min slices were transferred to normal aCSF at 21°C until they were triturated. A slice was then transferred to HEPES-buffered Dulbecco's Modified Earls Medium (DMEM) containing 0.1 mM leupeptin. The L-M was isolated with razor cuts under a dissecting microscope, transferred to a test tube containing 1 ml of the modified DMEM, and dispersed by trituration with fire-polished Pasteur pipettes. The whole solution was then transferred to the recording perfusion chamber mounted on an inverted microscope (IM-35, Zeiss).

Cell perfusion. The interneurons were allowed to settle on a poly-l-lysine coated coverslip for 10 min before the DMEM was replaced by superfusion of control aCSF (21°C) containing sucrose (40 mM), tetrodotoxin (TTX, 1.0 µM), 4-aminopyridine (1 mM), and tetrathyrammonium (10 mM) (pH 7.35; 280 mM). The recording solution was aerated with 95% O₂/5% CO₂, and the rate of superfusion was adjusted to allow complete exchange of chamber solution within 60 sec. High-resistance seals (>2 GΩ) were obtained from selected interneurons, the membrane was ruptured with negative pressure, and the cell contents were exchanged with the pipette-filling solution (in mM): 120 CsF, 15 HEPES, 10 EGTA, 2 Mg-ATP, and 2 TEA-acetate; (pH 7.2; 280 mM). Experiments began several minutes after breakthrough, once the LTT Ca²⁺ current was isolated and the holding current was stable.

Electrophysiological recording. Patch electrodes were pulled from 1.0 mm o.d. thin-walled glass (1B 100F, World Precision Instruments) in two stages on a Narishige puller (PP-83). Electrodes were filled with the intracellular solution (described above) and had a tip resistance of 5-10 MΩ when tested in recording solution. A 7.5 mV liquid-junction potential was measured between the electrode and bath solution; however, voltage command levels were not compensated (thus, command potentials were shifted 7.5 mV in the positive voltage direction). Current recordings were observed using a single-electrode voltage-clamp amplifier (Axopatch-1A) and were filtered at 5 KHz. Compensation circuitry was used to minimize potential series resistance errors and could compensate 60-80% of the series resistance. Data were digitized and analyzed using pClamp software (Axon Instruments). Leak and capacitance currents were eliminated by subtracting currents from a holding potential of -60 mV (where the LTT Ca²⁺ current was inactivated) from currents obtained at a holding potential of -100 mV (where inactivation was minimal).

Materials. All inorganic salts were purchased from Fisher. Chemicals utilized were trypsin (Sigma, T-1005), hyaluronidase (Sigma, H-1254), kynurenic acid (Sigma, K-3735), leupeptin (Sigma, L-2884), tetradotoxin (Sigma, T-8024), 4-aminopyridine (Sigma, A-0152), carbachol (CH; Sigma, C-4382), atropine (Sigma, A-0257), 5-hydroxytryptamine (5-HT; Sigma, H-3755), suxamethonium (Sigma, S-7393), and baclofen (CIBA-Geigy). All chemicals were added hyperosmotically to the recording solution from concentrated stocks.

Results

Dissociation of L-M cells

L-M interneurons had a diverse morphology after dissociation with typical examples shown in Figure 1. The cells were easily recognized, and their morphology was very similar to that observed when L-M cells were stained by intracellular injection of Lucifer yellow and HRP (Misgeld and Frotscher, 1986; Kagawachi and Hama, 1987; Kunkel et al., 1988; Lacaille and Schwartzkroin, 1988a). Most cells were fusiform, being multi-polar with several primary dendritic branches still intact. Cell body size ranged from 13 to 20 µm in diameter. The two other main cell types in the CA1 hippocampal region are astrocytes and CA1 pyramidal cells. Astrocytes were easily discerned by their smaller cell bodies (10-12 µm) and numerous processes (S. Duffy et al., 1991). CA1 pyramidal cells were observed only when the whole CA1 region was dissociated. Their morphology was different in that they typically had a pyramidal cell body and one apical dendritic process left after dissociation (Kay and Wong, 1986, 1987). Therefore, the main criteria that the cells voltage-clamped in this study were indeed L-M interneurons were that (1) only the L-M region was dissociated and (2) the morphologies of dissociated interneurons were distinct from pyramidal cells and were similar to previous reports of their morphology revealed by injections of intracellular markers.

**LTT Ca²⁺ currents**

These experiments were designed to examine the presence of calcium currents in L-M interneurons. The electrode contained Cs⁺ to block K⁺ currents and F⁻, which has been shown to suppress high-threshold Ca²⁺ currents (Kay et al., 1986). Whole-cell voltage-clamp recordings were obtained from >200 cells, but only 68 met the criteria for inclusion in this study (<40 pA holding current at -100 mV holding potential). Input resistance was measured in a smaller number of these cells and was 2.68 ± 0.36 GΩ (mean ± SEM; n = 10). All cells that were voltage-clamped from the L-M region exhibited a pronounced inward current that was greater than 2 nA in control solution. This inward current was totally blocked by TTX (0.5-1 µM) and was most likely a voltage-activated sodium current. After the sodium current was blocked with TTX, a transient inward current was observed in all cells (n = 68), including the other cells.
(>200) that had high or unstable holding currents. This inward current typically activated at -60 mV with a peak amplitude of 100 ± 9 pA (±SEM; n = 15) at -30 mV (Fig. 2). The current was totally inactivated at a holding potential of -60 mV. High-threshold Ca²⁺ currents were sometimes observed in these cells immediately following rupture of the membrane. However, the high-threshold Ca²⁺ current quickly deteriorated when pipettes contained F⁺. Extracellular Cd⁺⁺ (<25 μM) totally blocked any remaining high-threshold Ca²⁺ currents without altering the LTT Ca²⁺ currents.

Ca²⁺ appeared to be the major charge carrier for the inward current. Figure 3 shows the alteration in inward current that was observed when external Ca²⁺ was reduced. A decrease in extracellular Ca²⁺ from 2 to 0.1 mM suppressed the inward current. The current reappeared when the concentration of Ca²⁺ was increased to control levels of 2 mM.

Kinetics of the LTT Ca²⁺ current

The voltage dependence of steady state inactivation and activation of the LTT Ca²⁺ current is shown in Figure 4. Steady state inactivation was measured by varying the holding potential pulse from -120 to -45 mV and activating peak current with a command potential to -30 mV. Steady state inactivation was observed when the holding potential was positive to -100 mV. The current was half-inactivated at -84 mV and was totally inactivated at -60 mV. The fractional current, normalized to the maximum current, was smoothly voltage dependent and was fit with the following Boltzmann equation:

\[
\frac{I}{I_{\text{max}}} = 1 + \exp\left(\frac{V - V_{0.5}}{k}\right)^{-1},
\]

where \(I\) is peak current at \(V\), \(I_{\text{max}}\) is the maximum current from a holding potential of -100 mV, \(V\) is the holding potential, \(V_{0.5}\) is the half-inactivation value, and \(k\) is the steepness factor. The curve was best fit when \(V_{0.5} = -84.3\) mV and \(k = 6.3\). The voltage dependence of activation of the LTT Ca²⁺ current was examined by varying command potential pulses from a constant holding potential of -120 mV. The current was half-activated at -47 mV. The curve was smoothly voltage dependent and was fit by the Boltzmann function

\[
\frac{I}{I_{\text{max}}} = 1 + \exp[-(V - V_{0.5})/k]^{-1},
\]
Figure 2. LTT Ca$^{2+}$ currents recorded in an acutely dissociated L-M interneuron. A, Whole-cell voltage clamping revealed the LTT Ca$^{2+}$ current when the cell was clamped at a holding potential of -100 mV. Averaged currents (n = 4) are illustrated. Command potentials (Cp) more positive than -70 mV activated the LTT Ca$^{2+}$ current. Peak inward current was recorded at -30 mV. The broken lines indicate 0 current. B, The I/V relationship of the LTT Ca$^{2+}$ current is plotted. Points of the plot are the mean ± SEM (n = 15 separate cells). Peak current of 100 ± 9 pA was recorded at -30 mV.

The best fit of the curve was obtained when $V_{50} = -47.5$ mV and $k = 5.5$.

The time-to-peak of the LTT Ca$^{2+}$ current ($t_{50}$) was voltage dependent, and it ranged from 10 msec at -60 mV to 4 msec at 0 mV (Fig. 5). The rate constant of inactivation of the current ($r_1$) was also voltage dependent and ranged from 27 msec at 60 mV to approximately 12 msec at > -30 mV. The rate of inactivation of the LTT Ca$^{2+}$ current was estimated using an iterative fitting program (CLAMPFIT, Axon Inst.). In all cases the rate of inactivation was best fit by a single exponential ($R > 0.95$), indicating that there was no contamination by other Ca$^{2+}$ current subtypes under these conditions.

The recovery from inactivation was examined by repolarizing cells to -100 mV from a holding potential of -60 mV for various latencies before eliciting the inward current with a command pulse to -30 mV (Fig. 6). Recovery to 90% occurred within 200 msec. These properties are very similar to those reported for LTT Ca$^{2+}$ currents in many other cell types.

The block of LTT Ca$^{2+}$ currents by lanthanum and divalent cations in other cell types has a unique selectivity and occurs at lower concentrations of Ni$^{2+}$ than of Cd$^{2+}$. We did not find a similar differential sensitivity (Fig. 7). The relative potency of block was as follows: (IC$_{50}$ in mM; mean ± SEM): La$^{3+}$ (2.9 ± 0.3 x 10$^{-5}$; n = 3) > Zn$^{2+}$ (1.5 ± 0.12 x 10$^{-4}$; n = 3) > Cd$^{2+}$ (2.6 ± 0.20 x 10$^{-4}$; n = 5) > Ni$^{2+}$ (4.0 ± 0.31 x 10$^{-4}$; n = 5) > Co$^{2+}$ (7.6 ± 0.30 x 10$^{-4}$; n = 3). The depression at each concentration was observed after a 2 min application. It is interesting to note that in this time frame Cd$^{2+}$ is more potent at blocking the LTT Ca$^{2+}$ current than is Ni$^{2+}$. This has also been observed in cells from the hypothalamus (Akaike et al., 1989).

Figure 3. Ca$^{2+}$ dependency of the LTT Ca$^{2+}$ current. A voltage step to -30 mV from a holding potential of -100 mV activated the LTT Ca$^{2+}$ current in control solution containing 2 mM Ca$^{2+}$. When extracellular Ca$^{2+}$ was reduced to 0.1 mM, the LTT Ca$^{2+}$ was depressed but recovered when extracellular Ca$^{2+}$ was returned to control levels of 2 mM.

Figure 4. Voltage dependency of inactivation and activation of the LTT Ca$^{2+}$ current. The inactivation curve [left curve (□)] was analyzed by a changing holding potential from -120 to -45 mV and evoking peak current with a command potential to -30 mV. The points plotted are mean ± SEM from measurements in four different cells. The points were fit by a Boltzmann equation as described in Results. The voltage at which half-maximal inactivation occurred was -84 mV, and the steepness factor of the equation (k) was 6.3. The voltage dependency of activation [right curve (●)] was analyzed by stepping to command potentials from -85 to -15 mV from a holding potential of -120 mV. The points plotted are mean ± SEM from five different cells. The data points were fit by the Boltzmann relation as described in Results. The voltage at which half-maximal activation occurred was -47 mV, and the steepness factor was 5.5.
Modulation by neurotransmitters

Application of CCH or 5-HT enhanced the amplitude of the LTT Ca\(^{2+}\) current. CCH (50 µM) increased the peak amplitude of the LTT Ca\(^{2+}\) current by 54 ± 8% (mean ± SEM; n = 18 out of 22 cells; Fig. 8). Neither the activation threshold nor holding current was altered. Desensitization of the response to CCH did not occur in the time course of the experiments (within a few minutes). The enhancement of the current by CCH was rapidly reversed following washout and could be evoked repetitively with reapplication of the drug. The enhancement of the LTT Ca\(^{2+}\) current by carbachol was blocked by atropine (1 µM; n = 9). Therefore, the response appears to be mediated by muscarinic receptors. We did not further differentiate between muscarinic receptor subtypes.

5-HT (30 µM) increased the LTT Ca\(^{2+}\) current by 61 ± 11% (mean ± SEM; n = 19 out of 22 cells; Fig. 9) without affecting holding current. However, in the presence of 5-HT, the peak current was often observed at a more negative potential. The enhancement of the peak LTT Ca\(^{2+}\) current occurred rapidly after application of 5-HT, and there was no evidence for desensitization during application for several minutes. The action of 5-HT was quickly reversible following washout and could be evoked repetitively with reapplication of the drug. Co-application of spiperone (1 µM; n = 7), a serotonergic antagonist, totally blocked the 5-HT-induced enhancement of the LTT Ca\(^{2+}\) current.

Synaptic inputs from the septum onto L-M interneurons have also been shown to contain GABA (Freund and Antal, 1988). We examined the action of baclofen, a GABA\(_{\text{A}}\) agonist, on the LTT Ca\(^{2+}\) current. Baclofen (20 µM) reversibly depressed the magnitude of the LTT Ca\(^{2+}\) current by 53 ± 16% (mean ± SEM; n = 5 out of 7 cells; Fig. 10).
Figure 7. Block of the LTT Ca\(^{2+}\) current by lanthanum and divalent cations [La\(^{3+}\) (O), Zn\(^{2+}\) (\(\blacktriangledown\)), Cd\(^{2+}\) (\(\bullet\)), Ni\(^{2+}\) (\(\blacktriangle\)), and Co\(^{2+}\) (\(\bigcirc\))]. The fractional depression of the LTT Ca\(^{2+}\) current plotted as a function of the different concentrations of the indicated ions. The peak currents were evoked by command potentials to \(-25\) mV from a holding potential of \(-100\) mV. The points plotted for each ion are representative of individual experiments.

Discussion

In a previous study, Lacaille and Schwartzkroin (1988a) suggested that L-M interneurons might have low-threshold Ca\(^{2+}\) spikes. They reported that when the cells were hyperpolarized the firing response to a depolarizing current pulse changed from steady tonic firing to phasic burst firing. Interneurons in the oriens-alveus or pyramidal layer do not exhibit this transition in firing pattern (Lacaille and Williams, 1990). The present study on acutely dissociated L-M interneurons confirms that these cells do indeed have an LTT Ca\(^{2+}\) current, which could underlie a low-threshold Ca\(^{2+}\) spike. This current fit the criteria for a t-type Ca\(^{2+}\) current because (1) it was transient and activated at a low threshold, (2) it was insensitive to TTX but was Ca\(^{2+}\) dependent, (3) it was totally inactivated at \(-60\) mV holding potential, and (4) it displayed metabolic stability in that it did not “run down” significantly in the time course of these experiments.

Analysis of the kinetic properties of the LTT Ca\(^{2+}\) current in L-M interneurons indicates many similarities to this type of Ca\(^{2+}\) current in other cells (e.g., Carbone and Lux, 1984b; Ryu and Randic, 1990) but also some important differences. A major difference is that the time-to-peak and the rate of inactivation are faster in the present study. For example, the time-to-peak and time constant of inactivation over a range of potentials have been reported to be \(>7\) msec and \(>20\) msec, respectively, in LGN neurons (Hernandez-Cruz and Pape, 1989) and \(>10\) msec and \(>40\) msec, respectively, in hypothalamic neurons (Akaike et al., 1989). However, the concentration of external Ca\(^{2+}\) in these studies was 5 and 10 mM, whereas the currents in the present study were recorded in 2 mM Ca\(^{2+}\), which is much closer to the extracellular concentration in vivo. Increasing the concentration of extracellular Ca\(^{2+}\) alters the voltage sensitivity of most currents by charge screening effects and also slows the activation and inactivation kinetics for Ca\(^{2+}\) currents (Ohmori and Yoshii, 1977; Wilson et al., 1983). Although we did not examine the time constants in different Ca\(^{2+}\) concentrations, this does seem to be a reasonable explanation for the differences.

The kinetic analysis also gives some clues as to the effect that this current might have on L-M interneuron firing pattern. These cells have a resting potential of approximately \(-60\) mV (Lacaille and Schwartzkroin, 1988a), which would inactivate the LTT Ca\(^{2+}\) current. Hyperpolarization that lasted \(>50\) msec would cause significant deinactivation (\(>60\%\)) of the current so that a low-threshold Ca\(^{2+}\) spike may fire as rebound excitation. This pattern has been reported in the inferior olive (Llinas and Yarom, 1981) and the thalamus (Jahnsen and Llinas, 1984a).

Figure 8. Enhancement of the LTT Ca\(^{2+}\) current by muscarinic stimulation. A. Illustrated LTT Ca\(^{2+}\) currents were evoked from a holding potential of \(-100\) mV to the indicated command potentials (\(Cp\)). The smaller-amplitude currents at each potential are the control currents, and the enhanced currents are those recorded in CCH (50 \(\mu\)M). B. The \(I/V\) relationship of the LTT Ca\(^{2+}\) current (same cell as A) is illustrated showing the enhancement in the amplitude but no shift in the \(I/V\) relationship induced by CCH. The enhancement of the current in CCH was reversible upon washout. C. The peak inward current elicited by a command potential of \(-25\) mV from a holding potential of \(-100\) mV is plotted as a function of time. Perfusion of CCH (30 \(\mu\)M) quickly and reversibly enhanced the amplitude of the current from 100 to 175 pA. However, in the presence of atropine (Atr; 1 \(\mu\)M), CCH had no effect. This indicates that CCH was acting on a muscarinic receptor.
The LTT $\text{Ca}^{2+}$ current has been shown to be stable in the presence of intracellular $\text{F}^-$ in several cell types (Hernandez-Cruz and Pape, 1989; Takahashi et al., 1989), so it was not surprising that the LTT $\text{Ca}^{2+}$ current in L-M interneurons was also stable when the pipette contained $\text{F}^-$. What is surprising is that modulation of the LTT $\text{Ca}^{2+}$ current could still be observed. GTP-binding proteins, which mediate signal transduction by interacting with receptor proteins, are activated by $\text{F}^-$ in the presence of $\text{Al}^3+$ (Sternweis and Gilman, 1982; Bigay et al., 1985). The activation of GTP-binding proteins results from the binding of $\text{AIF}_\gamma$ to the nucleotide site of $G_\gamma$, next to the $\beta$-phosphate of GDP, mimicking the GTP $\gamma$-phosphate (Kanaho et al., 1985; Bigay et al., 1987). The dissociation of $G_\gamma$ from $\beta\gamma$ is thereby enhanced. Nevertheless, modulation of the LTT $\text{Ca}^{2+}$ current by neurotransmitters was reversible following washout and reproducible with reapplication of the drug. These two observations suggest that maximal activation of GTP-binding proteins, under our conditions, did not occur.

L-M interneurons are believed to be inhibitory because (1) they stain positive for glutamic acid decarboxylase (Ribak et al., 1978; Kosaka et al., 1985), the enzyme indicative of GABAergic neurons, (2) they form symmetrical synapses upon hippocampal dendrites indicative of inhibitory synapses (Kunkel et al., 1988), and (3) simultaneous intracellular recordings between L-M interneurons and pyramidal cells have provided some indication of inhibition (Lacaille and Schwartzkroin, 1988b). The presence of low-threshold $\text{Ca}^{2+}$ spikes and LTT $\text{Ca}^{2+}$ currents in L-M interneurons could have significant implications concerning the generation of different firing patterns in hippocampal pyramidal neurons. Low-threshold $\text{Ca}^{2+}$ spikes result in rhythmic bursting in neurons in the thalamus (Deschenes et al., 1984; Jahnssen and Llinas, 1984a,b) and the lateral habenula (Wilcoxon et al., 1988) at approximately 10 Hz, which is the theta frequency in the hippocampus. L-M interneurons normally fire action potentials at a slow, steady rate (Kawaguchi and Hama, 1987). Enhancement of the LTT $\text{Ca}^{2+}$ current and membrane hyperpolarization could cause L-M cells to shift from tonic firing into a repetitive bursting mode. It is possible that burst firing in L-M interneurons could alter the strength of inhibition on pyramidal cells from a steady tonic level to rhythmically varying. Thus, membrane potential oscillations in L-M interneurons could cause rhythmic activity in hippocampal pyramidal cells.

Cholinergic and GABAergic inputs to the hippocampus arise from the medial septum (Storm-Mathisen, 1977), some of which have been shown to synapse directly upon L-M interneurons (Freund and Antal, 1988). Serotonergic inputs to the hippocampus arise from the raphe nuclei, and their innervation has been shown to be particularly dense in the L-M region (Storm-Mathisen, 1977). A recent study, utilizing electron microscopy, has also shown that serotonergic inputs from the raphe make synaptic connections with L-M interneurons (Freund et al., 1990). Both the cholinergic-septal and the serotonin-raphe pathways are believed to be involved in generating type 1 and type 2 theta rhythm in the hippocampus (Bland, 1986; Vanderwolf and Baker, 1986). Generation of cholinergic-induced theta rhythm in the hippocampus does not appear to depend upon rhythmic synaptic inputs from structures outside the hippocampus because perfusion of carbachol on isolated brain slices can induce theta rhythm (Konopacki et al., 1987; MacVicar and Tse, 1989). It appears, therefore, that synaptic circuitry and intrinsic membrane conductances sufficient for generating theta are intrinsic to the hippocampus. The synaptic effects of cholinergic and serotonergic inputs on hippocampal pyramidal neurons are quite different (reviewed in Nicoll, 1988). Cholinergic inputs depress high-threshold $\text{Ca}^{2+}$ currents and depolarize pyramidal cells by blocking the M-current. Serotonergic inputs hyperpolarize pyramidal neurons by increasing potassium conductance. Serotonin has also been reported to enhance $\text{Ca}^{2+}$ spikes in the substantia nigra (Nedergaard et al., 1988) and to enhance LTT $\text{Ca}^{2+}$ currents in rat spinal motoneurons (Berger and Takahashi, 1990). However, both transmitter systems ap-
appear to have a common effect on L-M interneurons of increasing LTT Ca$^{2+}$ currents. It has previously been suggested that the septal pacemaker drives hippocampal theta by rhythmically activating interneurons (Buzsaki et al., 1983; Stewart and Fox, 1990). Interneurons have also been reported to be theta cells and fire at theta frequencies during behaviorally evoked theta (reviewed in Buzsaki et al., 1983; Fox et al., 1986). Our work suggests an alternative hypothesis: that cholinergic and serotonergic inputs may enhance LTT Ca$^{2+}$ currents, which could trigger endogenous oscillation of L-M interneurons and thereby induce hippocampal theta. GABAergic input may play a role in terminating theta by depressing the LTT Ca$^{2+}$ current.

In the CA3 region of isolated slices, we have shown that the CCH-induced theta rhythm is not affected by GABA$_A$ or GABA$_B$ antagonists but is blocked by kynurenic acid, a wide-spectrum excitatory amino acid antagonist (MacVicar and Tse, 1989). In the CA3 region, therefore, EPSPs underlie theta activity, possibly through glutamatergic input to pyramidal neurons. If L-M interneurons generate theta in the CA1 region, one would expect a much more prominent role for inhibitory circuits in generating theta in the slice. It is possible that there are multiple cellular components that generate theta that can all oscillate at 10 Hz. Synchronization could be due to both recurrent excitatory and inhibitory circuits.

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