Dietary Prenatal Choline Supplementation Alters Postnatal Hippocampal Structure and Function

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Li, Qiang, Shirley Guo-Ross, Darrell V. Lewis, Dennis Turner, Aaron M. White, Wilkie A. Wilson, and H. Scott Swartzwelder. Dietary prenatal choline supplementation alters postnatal hippocampal structure and function. J Neurophysiol 91: 1545–1555, 2004. First published November 26, 2003; 10.1152/jn.00785.2003. Choline, a compound present in many foods, has recently been classified as an essential nutrient for humans. Studies with animal models indicate that the availability of choline during the prenatal period influences neural and cognitive development. Specifically, prenatal choline supplementation has been shown to enhance working memory and hippocampal long-term potentiation (LTP) in adult offspring. However, the cellular mechanisms underlying these effects remain unclear. Here we report that choline supplementation, during a 6-day gestational period, results in greater excitatory responsiveness, reduced slow afterhyperpolarizations (sAHPs), enhanced afterdepolarizing potentials (ADPs), larger somata, and greater basal dendritic arborization among hippocampal CA1 pyramidal cells studied postnatally in juvenile rats (20–25 days of age). These data indicate that dietary supplementation with a single nutrient, choline, during a brief, critical period of prenatal development, alters the structure and function of hippocampal pyramidal cells.

INTRODUCTION

Choline is an essential nutrient for animals and humans. Its adequate supply during fetal development is critical, and dietary supplementation during this time could provide long-lasting benefits. Although choline is available in many foods, the normal dietary sources may prove to be insufficient during times of heightened need such as pregnancy and lactation (Zeisel and Blusztajn 1994). Therefore the recommended daily intake of this compound is higher during pregnancy and lactation (Blusztajn 1998; Zeisel and Blusztajn 1994).

Recent studies with animal models have indicated that prenatal choline supplementation does indeed produce enduring enhancements of CNS function. For example, prenatal choline supplementation has been shown to increase phospholipase-D activity in the hippocampus of adult offspring (Holler et al. 1996) and to decrease acetylcholine esterase (AChE) activity in the hippocampus of juvenile offspring (Cermak et al. 1999). These changes are consistent with the improvements in spatial transmission (Montoya and Swartzwelder 2000) and long-term potentiation (LTP) (Pyapali et al. 1998) in hippocampal slices from prenatally choline-supplemented rats.

These findings suggest that prenatal choline supplementation results in enhanced physiological functioning by hippocampal pyramidal cells. However, this has not been demonstrated at the cellular level, and the cellular mechanisms that underlie these changes are unknown. One possibility is that increased choline availability during early development could result in morphological or neurophysiological alterations in hippocampal pyramidal cells that promote cellular activity, since choline is a critical for membrane development. To test this hypothesis directly, we provided dietary choline supplementation to pregnant rat dams during a 6-day period from embryonic day E12 to E17, and measured membrane properties of CA1 pyramidal cells from juvenile rats using whole cell patch-clamp techniques in acute brain slices. In addition to assessing electrophysiology of CA1 pyramidal cells from both groups, CA1 pyramidal cells were also filled with the intracellular dye, biocytin, and morphological characteristics of dendrites and soma of biocytin-filled CA1 pyramidal cells were evaluated as well. We found that prenatal choline supplementation altered both the electrophysiology and morphology of CA1 pyramidal cells.

METHODS

Prenatal choline treatment

Pregnant, Sprague-Dawley albino rats were obtained (Charles River, Kingston, NY) at day 9 of gestation (E9). Animals were housed individually in clear polycarbonate cages (27.9 × 27.9 × 17.8 cm3), and food and water were provided ad libitum with a 12-h light/dark cycle. They were fed food that consisted of purified formula AIN-76A diet (Dyets, Bethlehem, PA) containing 7.9 mmol/kg choline chloride and water. Prenatal choline treatment was carried out from days E12 to E17. The dams were divided into control and choline supplemental groups. Control dams received an AIN-76A diet containing 7.9 mmol/kg choline chloride and water sweetened with 50 mM saccharine. Dams in the supplemented group received the same purified formula of AIN-76A as the control rats, but they also received water containing 25 mM choline chloride sweetened with 50 mM saccharine. Saccharine was used to neutralize the bitter taste of choline in the diet and to equalize the water intake among dams in the two treatment groups. The average daily food intake was calculated for each dam.
The dams in the control group consumed a mean of 1.3 mmol/kg/day of choline (from food source only). Dams in the supplemented group consumed a mean of choline of 4.6 mmol/kg/day (from food and water sources combined). This exposure paradigm was similar to that originally described by Meck et al. (1989). The 25 mM choline chloride concentration has been accepted as a standard treatment and has been associated with improvement of cognition in adult offspring (Meck et al. 1989), protection of neocortical cells against neurotoxicity-induced by MK-801 (Guo-Ross et al. 2002, 2003), and protection against seizure activity (Yang et al. 2002). The 25 mM choline solution causes an approximately four times higher intake of choline by the pregnant dam than that provided by the AN76A diet, and it is not outside of the dietary variation observed in human population. After day 17 of gestation, all animals were fed normal AN76A diet containing 7.9 mmol/kg choline chloride and saccharine-free water. At birth the pups were cross-fostered to untreated foster dams and were killed as juveniles, between postnatal days 20 and 25, for harvesting of hippocampal slices. All of the offspring used in this study were male. All methods were approved by the Institutional Review Boards at both Duke University and the Durham VA Medical Center.

### Hippocampal slice preparation

In all instances, the experimenters were blind to the treatment condition of the animals before death and to cell status during recording and analyses (control vs. choline supplementation). The rats were anesthetized with isoflurane and decapitated. The brains were quickly removed from the skulls and placed in cold (4°C) artifical spine fluid (ACSF) containing (in mM) 120 NaCl, 3.3 KCl, 1.23 NaH₂PO₄, 25 NaHCO₃, 1.2 MgSO₄, 1.8 CaCl₂, and 10 D-glucose at pH 7.3, previously saturated with 95% O₂-5% CO₂. Coronal slices (300 μm thick) were prepared on a vibratome (Leica VT 1000S) and were incubated in a holding chamber containing normal ACSF that was visible apical dendrites. Tight seals (1G° pH 7.3, previously saturated with 95% O₂-5% CO₂) were obtained on CA1 pyramidal neurons using a waga-puller (Model P-97, Sutter Instrument, Novato, CA). The pipettes were pulled from borosilicate glass capillary tubing (1.5 mm OD, 1.05 mm ID, World Precision Instruments, Sarasota, FL) on a Flaming-Brown horizontal microelectrode puller (Model P-97, Sutter Instrument, Novato, CA). The pipettes were filled with an intracellular solution containing (in mM) 120 NaCl, 3.3 KCl, 1.23 NaH₂PO₄, 25 NaHCO₃, 1.2 MgSO₄, 1.8 CaCl₂, and 10 D-glucose at pH 7.3, previously saturated with 95% O₂-5% CO₂. Coronal slices (300 μm thick) were prepared on a vibratome (Leica VT 1000S) and were incubated in a holding chamber containing normal ACSF that was continuously bubbled with 95% O₂-5% CO₂ at room temperature (22-24°C).

### Whole cell voltage-clamp recording

Our whole cell patch-clamp techniques have been described in detail previously. Patch pipettes were pulled from borosilicate glass capillary tubing (1.5 mm OD, 1.05 mm ID, World Precision Instruments, Sarasota, FL) on a Flaming-Brown horizontal microelectrode puller (Model P-97, Sutter Instrument, Novato, CA). The pipettes were filled with an intracellular solution containing (in mM) 130 K-glucanate, 7 KCl, 10 HEPES, 4 Mg-ATP, and 0.2 tris-GTP. pH was adjusted to 7.25, and osmolarity was adjusted to around 280 mOsm. The pipette resistances were in the range of 4–7 MΩ. In some recordings, 0.3–0.4% biocytin (Sigma Chemical, St. Louis, MO) was added to the intracellular solution for later visualization of the morphology of the recorded cells. After 1 h of incubation in the holding chamber, the slice was transferred into a small submersion recording chamber that was maintained at room temperature (22–24°C) and contained the ACSF described above. It was secured in place with a bent piece of platinum wire and was continuously bathed with ACSF. Individual cells were visualized using an infrared differential interference contrast (IR-DIC) Zeiss Axiostkop microscope and a 40× water immersion objective. To be targeted for recording, we required that neurons be located 70 μm below the surface of the slice and have pyramidal-shaped somata with visible apical dendrites. Tight seals (>1 GΩ) were obtained on CA1 pyramidal cells, and whole cell recordings were made after rupturing the cell membrane with gentle suction. Whole cell current-clamp recordings were made using an Axopatch 1-D amplifier (Axon Instruments, Foster City, CA) and Strathclyde Electrophysiology Software, Whole Cell Program for Window (WINWCP) with an interface (BNC-2090, National Instruments, Austin, TX) to a PC-based computer.

### Analysis and measurement of electrophysiological parameters

Electrophysiological properties of the neurons were carried out off-line with the WINWCP program. Since most of the parameters being investigated were voltage dependent, resting membrane potentials were maintained at −64 to −66 mV throughout the experiments. The current-voltage relations were obtained by injecting 600-ms hyperpolarizing current steps (range, −200 to +200 pA). The input resistance (Rin) of each neuron was calculated from the steady-state voltage deflection during the last 80 ms of 600-ms hyperpolarizing current steps. The membrane time constant was determined by fitting the voltage transient deflection (<10 mV) by a 60-pA hyperpolarizing current injection to a single order exponential function. The ratio of “Sag” was calculated by dividing the steady-state voltage by the peak voltage in response to 600-ms hyperpolarizing current steps (range, −20 to −200 pA). The afterhyperpolarizing potential (AHP) was studied using a 100-ms depolarizing current that reliably elicited a train of four action potentials. The amplitude of the afterdepolarizing potential (ADP) was measured from the point where the repolarizing potential began to depolarize to its maximal potential. This result in amplitude measurements generally in the 1.0–4.0 mV range.

The action potential threshold was determined by measuring voltage deflection from the resting membrane potential to the beginning of an action potential. The action potential amplitude was measured from the threshold to the peak, and the duration was measured at the half-amplitude. Spike frequency adaptation (SFA) was studied using 600-ms depolarizing current pulses (range, +20 to +200 pA) and was determined by measuring the instantaneous discharge frequency. The extent of SFA was expressed as the ratio of the frequency of the last two spikes to the frequency of the first two spikes and is termed the adaptation ratio. The number of action potentials produced by each neuron during the depolarizing pulse was also counted. Single action potentials were evoked by injecting 5-ms depolarizing current pulses at spike threshold intensity.

### Histological identification of CA1 pyramidal neurons

During recording, some neurons were filled with biocytin. After recording, those slices remained in the recording chamber for an additional 10–20 min to allow biocytin transport within the dendrites and axons. The slices were then placed in 4% paraformaldehyde (TBS) containing 1% H₂O₂ for 30 min. The slices were incubated with avidin-biotin-peroxidase complex (ABC kit, Vector Labs, Burlingame, CA) in TBS containing 0.05% Triton X-100 overnight at 4°C, rinsed three times in PBS, and reacted in a solution containing DAB (DAB kit, Vector Labs, Burlingame, CA). The slices were then cleared and mounted. The morphology of the biocytin-filled CA1 pyramidal cells was examined under a light microscope by a blinded observer, and neurons were drawn using a NeuroLucida setup, at 40× magnification, using unsectioned slices (Pyapali et al. 1998).

### Statistical analysis

Two-way ANOVAs were used with Student’s t-test as posthoc tests where appropriate. In instances in which the statistical inferences involved a simple comparison of two means, unpaired Student’s t-test was used. The significance level was set to P < 0.05 for all statistical tests. All grouped data are presented as means ± SE in the figures.

**RESULTS**

As reported previously (Guo-Ross et al. 2002, 2003), at the time when the rats were killed, there were no significant
differences in average food and water intake or body weight between the two treatment groups. Pregnant rats did not exhibit abnormal behaviors, and there were no differences in the size of litters or the survival rate of pups between the two groups. The animals from which hippocampal slices were made came from 15 control dams and 16 choline-supplemented dams. Three to four male offspring from each dam were used. A total of 103 CA1 hippocampal pyramidal neurons were recorded, of which 53 were from choline-supplemented rats. Three to four

**Morphological properties of CA1 pyramidal cells**

Biocytin was infused into the first 26 neurons from which whole cell recordings were made. Sixteen of these neurons were from control offspring \((n = 6)\) and 10 were from prenatally choline-supplemented offspring \((n = 4)\). All filled neurons were confirmed to be CA1 pyramidal cells and contained soma, basal dendrites, and a long apical dendrite with arbors at least extending into the stratum radiatum (Pyapali et al. 1998). In some recovered cells, axons were also observed. Figure 1A shows photomicrographs of three CA1 pyramidal cells from control \((\text{middle})\), and two from choline-supplemented \((\text{right})\) rats. The detailed Neurolucida reconstructions of some CA1 pyramidal cells filled with biocytin are shown in Fig. 1, B (control) and C (choline supplemented). After morphometric analysis using Neurolucida analysis, a total of nine cell parameters were quantified and are summarized in Table 1. There were a number of significant differences in morphological properties of CA1 pyramidal cells between the two treatment groups, suggesting that prenatal choline supplementation resulted in morphological alterations of CA1 pyramidal cells. The two-dimensional cross-sectional area and perimeter of CA1 somata from prenatally choline-supplemented animals were larger than those from controls \((t_{26} = 3.72, P < 0.005\) and \(t_{26} = 3.99, P < 0.003\), respectively). The number of primary and secondary basal dendrite branches of cells from prenatally choline-supplemented rats was also greater than those from controls \((P < 0.003\) and 0.005, respectively). Sholl analysis showed that prenatal choline supplementation caused a proliferation of primary basal dendrites. Figure 2A shows that the average number of basal dendritic intersections in the Sholl analysis was significantly higher among cells from prenatally choline supplemented rats \((F_{1,23} = 4.95, P = 0.04)\). The difference in the density of basal dendrite intersections was greatest within 140 \(\mu\)m of the soma. In contrast, prenatal choline supplementation appeared to have minimal effect on development of apical dendrites. For example, distance from soma to the first apical dendritic branch was almost identical between groups, and there was no significant difference in the

**TABLE 1. Morphological properties of CA1 pyramidal cells recorded from control and choline supplemented rats**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>n</th>
<th>Supplementation</th>
<th>n</th>
<th>(P \text{ value})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soma minimum diameter ((\mu)m)</td>
<td>14.3 ± 0.45</td>
<td>16</td>
<td>15.9 ± 0.75</td>
<td>10</td>
<td>0.0612</td>
</tr>
<tr>
<td>Soma maximum diameter ((\mu)m)</td>
<td>16.9 ± 0.29</td>
<td>16</td>
<td>19.9 ± 0.53</td>
<td>10</td>
<td>0.0011</td>
</tr>
<tr>
<td>Soma area ((\mu)m(^2))</td>
<td>154.3 ± 12.01</td>
<td>16</td>
<td>219.8 ± 14.64</td>
<td>10</td>
<td>0.0022</td>
</tr>
<tr>
<td>Soma perimeter ((\mu)m)</td>
<td>50.2 ± 2.77</td>
<td>16</td>
<td>64.2 ± 1.79</td>
<td>10</td>
<td>0.0012</td>
</tr>
<tr>
<td>Total length of apical dendrites ((\mu)m)</td>
<td>1379.3 ± 198.2</td>
<td>16</td>
<td>1247.1 ± 130.2</td>
<td>10</td>
<td>0.6307</td>
</tr>
<tr>
<td>Total length of basal dendrites ((\mu)m)</td>
<td>4512.5 ± 417.9</td>
<td>16</td>
<td>5019.5 ± 429.2</td>
<td>10</td>
<td>0.4223</td>
</tr>
<tr>
<td>Number of primary basal dendrites</td>
<td>4.3 ± 0.23</td>
<td>16</td>
<td>6.2 ± 0.42</td>
<td>10</td>
<td>0.0027</td>
</tr>
<tr>
<td>Number of secondary basal dendrites</td>
<td>10.5 ± 0.79</td>
<td>16</td>
<td>14.9 ± 0.53</td>
<td>10</td>
<td>0.0047</td>
</tr>
<tr>
<td>Distance to first apical dendritic branch ((\mu)m)</td>
<td>60.3 ± 3.26</td>
<td>16</td>
<td>59.7 ± 8.74</td>
<td>10</td>
<td>0.9444</td>
</tr>
</tbody>
</table>

Values are mean ± SE.
mean number of intersections of apical dendrites $\leq 500$ $\mu$m from the soma between groups (Fig. 2B).

**Electrophysiological properties of CA1 pyramidal cells**

**Passive membrane properties.** The basic membrane properties of CA1 hippocampal pyramidal cells of both groups were determined at the resting membrane potential and are summarized in Table 2. In general, these were unaltered by prenatal choline supplementation.

Resting membrane potentials were determined after seal rupture, and CA1 neurons with resting membrane potentials of at least $-55$ mV were used in this study. The average resting membrane potential for CA1 pyramidal cells in control rats was $-66 \pm 4.97$ mV ($n = 50$), and the average resting membrane potential for CA1 pyramidal cells in choline-supplemented rats was $-65 \pm 6.08$ mV ($n = 53$). There was no significant difference in resting membrane potentials between the two treatment groups ($P > 0.05$, unpaired t-test). The membrane time constant of CA1 pyramidal cells between the two groups was also almost identical (Table 2, $P > 0.05$, unpaired t-test).

Intracellular injection of graded hyperpolarizing currents (600 ms duration), at the resting membrane potential, elicited voltage responses, and these responses were used to assess the input resistance, membrane time constant, and presence of hyperpolarization-activated conductance. Figure 3, A and B, shows the membrane responses of two cells during the hyperpolarizing current injections, and the current and voltage relationships (I-V curves) from the neurons is shown in Fig. 3C. Generally, there was a linear I-V plot in response to hyperpolarizing current injections in cells from both groups. The input resistance for each cell was calculated as the slope of the regression line plotted across the I-V curve. Interestingly, CA1 pyramidal cells from choline-supplemented rats had significantly lower input resistances than did those from control animals (Table 1, $P < 0.05$, unpaired t-test).

All neurons showed slow inward rectification, or sag, on injection of hyperpolarizing current pulses as shown in Fig. 3, D and E. Averaged sag ratios for CA1 pyramidal cells from control and supplemented rats were $0.79 \pm 0.09$ ($n = 25$) and $0.80 \pm 0.11$ ($n = 34$), respectively. There was no significant difference in sag ratio between the two groups ($P > 0.05$, unpaired t-test). To determine if sag was due to hyperpolarization-activated cationic conductance, or $I_{Ca}$, 5 mM CsCl was bath applied (Staff et al. 2000). After perfusion of 5 mM CsCl for 4–6 min, the slow inward rectification was completely abolished, as indicated by the absence of sag at the bottom of each trace (Fig. 3, D and E), and this was recovered after washout.

We observed a complete block of $I_{Ca}$ in all CA1 pyramidal cells

**TABLE 2. Passive membrane properties of CA1 pyramidal cells recorded from control and choline supplemented rats**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>n</th>
<th>Supplementation</th>
<th>n</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting membrane potential (mV)</td>
<td>$-66 \pm 4.97$</td>
<td>50</td>
<td>$-65 \pm 6.08$</td>
<td>53</td>
<td>0.264</td>
</tr>
<tr>
<td>Input resistance (MΩ)</td>
<td>$82.8 \pm 5.44$</td>
<td>34</td>
<td>$70.3 \pm 2.73$</td>
<td>37</td>
<td>0.044</td>
</tr>
<tr>
<td>Membrane time constant (ms)</td>
<td>$20.52 \pm 1.02$</td>
<td>33</td>
<td>$20.56 \pm 0.93$</td>
<td>31</td>
<td>0.981</td>
</tr>
<tr>
<td>Sag ratio</td>
<td>$0.79 \pm 0.09$</td>
<td>25</td>
<td>$0.80 \pm 0.11$</td>
<td>33</td>
<td>0.903</td>
</tr>
<tr>
<td>Input resistance (MΩ) in 5 mM CsCl</td>
<td>$157.1 \pm 14.1$</td>
<td>11</td>
<td>$142.7 \pm 10.2$</td>
<td>11</td>
<td>0.418</td>
</tr>
<tr>
<td>Membrane time constant (ms) in 5 mM CsCl</td>
<td>$54.4 \pm 2.06$</td>
<td>13</td>
<td>$50.3 \pm 1.73$</td>
<td>12</td>
<td>0.447</td>
</tr>
</tbody>
</table>

Values are mean $\pm$ SE.
tested. These findings indicate that CA1 pyramidal cells from choline-supplemented rats had a cesium sensitive sag that was similar to that observed in neurons from control rats. After blocking the sag with CsCl, input resistance and membrane time constant increased in both groups equally (Table 2).

ACTIVE MEMBRANE PROPERTIES. At the resting membrane potential, we observed no spontaneous action potentials. On injection of depolarizing currents, neurons from both groups displayed regular spiking activity, as shown in Fig. 4. None of the cells manifested action potential bursts under these conditions. Table 3 is a summary of the active membrane properties of CA1 pyramidal cells of the both groups.

Current-clamp recordings from CA1 pyramidal cells from control and choline-supplemented rats revealed no significant differences in spike threshold, rheobase, amplitude, or duration of the first, second, or third action potentials (Table 3). However, we found several intrinsic properties that were significantly different between two groups, suggesting there was an enhancement of cellular excitability of CA1 pyramidal cells from prenatally choline-supplemented rats.

Figure 4 shows the firing patterns of two CA1 pyramidal cells, one from each of the treatment groups, in response to a 600-ms depolarizing current injection. Under normal recording conditions, control neurons began to discharge rapidly when spike threshold was reached, and the firing rate diminished rapidly (spike frequency adaptation, Fig. 4A). However, the CA1 pyramidal cells from choline-supplemented rats fired more action potentials with less SFA (Fig. 4B). The differences in SFA between the two groups were further evaluated by plotting spike sequences against the instantaneous firing frequency for graded depolarizing currents injected into the cells, and the results are shown in Fig. 4, C and D. In addition, CA1 pyramidal cells from prenatally choline-supplemented rats produced more action potentials than did those from controls in

FIG. 3. Current-voltage relationships of CA1 pyramidal cells recorded from control (A) and prenatally choline-supplemented (B) rats. Top panels show voltage responses to hyperpolarizing currents injections. C: I-V plots of the same neurons shown in top panels. Input resistances were computed as the change in voltage at steady state (SS). Scale bar:100 ms, 5 mV. *Spontaneous postsynaptic potentials (SPPs): 5 mM cesium chloride blocks hyperpolarizing active currents ($I_h$) in neurons from control (D) and prenatally choline-supplemented (E) rats.
response to injected currents (Fig. 5; $F_{(1,69)} = 39.6, P < 0.0009$). There was also a significant interaction between treatment group and the amplitude of injected current ($F_{(8)} = 22.4, P < 0.0009$), indicating that there was an earlier spike frequency accommodation in CA1 pyramidal cells recorded from control rats ($n = 31$) than in those recorded from choline-supplemented rats ($n = 40$).

SFA was also calculated as the ratio of the frequency of the final two action potential spikes to the frequency of the first two spikes during the depolarizing pulse. We found that the ratio was significantly higher in the CA1 pyramidal cells from prenatally choline-supplemented rats ($0.31 \pm 0.01$) compared with controls ($0.24 \pm 0.01; t_{(16)} = 6.39, P < 0.001$), indicating greater sustainable excitability among neurons from prenatally choline-supplemented rats. This propensity toward less spike frequency adaptation during depolarization could account, in
part, for the enhancement of LTP induction that we have observed in hippocampal slices from prenatally choline supplemented rats (Pyapali et al. 1998).

Another index of neuronal excitability is the slow AHP (sAHP) that follows a train of action potentials (Baraban and Schwartzkroin 1997; Storm 1990). As shown in Fig. 6, A and B, at the holding potential of –66 mV, sAHPs were observed in cells from both groups. However, the amplitude of the sAHPs in neurons from prenatally choline-supplemented rats (Fig. 6B) was significantly smaller compared with those from controls (Fig. 6A; \( t_{16} = 2.4, P = 0.03 \)). Since AHPs are voltage dependent, their peak amplitude is influenced by the membrane potential. All sAHPs were recorded under the same holding potential of –66 mV. Figure 6C shows the mean of peak the sAHP of CA1 pyramidal cells from both groups (control, \( n = 33 \); supplemented, \( n = 35 \)). The average sAHPs in CA1 pyramidal cells from choline-supplemented rats was significantly smaller that of control rats (unpaired t-test, \( P < 0.05 \)). Since a calcium-dependent potassium conductance underlies the sAHP, which regulates the rate of neuronal firing, and is subject to modulation by many neuromodulators including choline (Storm 1990), this conductance may be of mechanistic significance.

When brief depolarizing current pulses (5 ms) were applied to the neurons, single action potentials were elicited and were followed by an ADP. As shown in Fig. 7, the amplitude of the ADP was significantly smaller in CA1 pyramidal cells from control rats (Fig. 7A, left) compared with those from prenatally choline-supplemented rats (Fig. 7A, middle). Figure 7B compares the mean amplitude of the ADP of CA1 pyramidal cells from both groups (control, \( n = 24 \); supplemented, \( n = 24 \)). The amplitude and duration of ADP in cells from prenatally choline-supplemented rats were significantly larger than were those from controls (unpaired t-test, \( P < 0.05 \)).

### TABLE 3. Action potential properties of CA1 pyramidal cells recorded from control and choline supplemented rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>( n )</th>
<th>Supplementation</th>
<th>( n )</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spike threshold (mV)</td>
<td>–43.5 ± 0.74</td>
<td>32</td>
<td>44.6 ± 0.75</td>
<td>32</td>
<td>0.145</td>
</tr>
<tr>
<td>Rheobase (pA)</td>
<td>76.2 ± 4.9</td>
<td>32</td>
<td>66.6 ± 4.2</td>
<td>32</td>
<td>0.069</td>
</tr>
<tr>
<td>Spike amplitude (mV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First spike</td>
<td>97.1 ± 1.8</td>
<td>32</td>
<td>94.2 ± 2.0</td>
<td>32</td>
<td>0.151</td>
</tr>
<tr>
<td>Second spike</td>
<td>93.6 ± 1.5</td>
<td>32</td>
<td>89.7 ± 1.8</td>
<td>32</td>
<td>0.061</td>
</tr>
<tr>
<td>Third spike</td>
<td>93.4 ± 1.2</td>
<td>32</td>
<td>90.1 ± 1.8</td>
<td>32</td>
<td>0.063</td>
</tr>
<tr>
<td>Spike duration (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First spike</td>
<td>3.22 ± 0.15</td>
<td>32</td>
<td>3.12 ± 0.09</td>
<td>32</td>
<td>0.288</td>
</tr>
<tr>
<td>Second spike</td>
<td>3.39 ± 0.12</td>
<td>32</td>
<td>3.29 ± 0.12</td>
<td>32</td>
<td>0.269</td>
</tr>
<tr>
<td>Third spike</td>
<td>3.57 ± 0.09</td>
<td>32</td>
<td>3.50 ± 0.10</td>
<td>32</td>
<td>0.306</td>
</tr>
<tr>
<td>Spike duration at half amplitude (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First spike</td>
<td>1.25 ± 0.06</td>
<td>32</td>
<td>1.22 ± 0.06</td>
<td>32</td>
<td>0.364</td>
</tr>
<tr>
<td>Second spike</td>
<td>1.58 ± 0.09</td>
<td>32</td>
<td>1.52 ± 0.08</td>
<td>32</td>
<td>0.303</td>
</tr>
<tr>
<td>Third spike</td>
<td>1.57 ± 0.08</td>
<td>32</td>
<td>1.50 ± 0.08</td>
<td>32</td>
<td>0.239</td>
</tr>
<tr>
<td>ADP (mV)</td>
<td>1.19 ± 0.08</td>
<td>24</td>
<td>3.84 ± 0.34</td>
<td>24</td>
<td>0.0008</td>
</tr>
<tr>
<td>ADP duration (ms)</td>
<td>4.37 ± 0.78</td>
<td>24</td>
<td>7.12 ± 0.82</td>
<td>24</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Values are mean ± SE. ADP, afterdepolarizing potential.

![FIG. 5. Mean ± SE numbers of action potentials in response to depolarizing current injections in CA1 pyramidal cells from prenatally choline-supplemented rats (\( n = 40 \)) was higher than that of CA1 pyramidal cells from control rats (\( n = 31 \)).](http://jn.physiology.org/10.220.33.3/24/2004.jn.org)
DISCUSSION

As an essential nutrient, choline has been recommended for men and women, including elevated intake recommendations for pregnant and lactating women (Blusztajn 1998). However, there is little information available regarding effect of acute or prenatal choline on neurons. The present results provide the first evidence for specific action of prenatal choline supplementation on cellular activity in the offspring and indicate that prenatal choline supplementation could alter the physiology of CA1 pyramidal cells by affecting their excitability and morphology. In this study, we have compared for the first time the intrinsic membrane and morphological properties of CA1 pyramidal cells recorded from control and prenatally choline-supplemented rats. Although CA1 pyramidal cells recorded from prenatally choline-supplemented rats possess generally similar intrinsic membrane and morphological properties to those from control rats, our results indeed revealed a number of alterations in neuronal excitability of CA1 pyramidal cells recorded from prenatally choline-supplemented rats. Moreover, our results indicate that moderate dietary supplementation with an essential nutrient during a brief period of prenatal development produces enduring changes in both the structure and function of hippocampal CA1 pyramidal cells. They are also unique in that most studies of prenatal dietary manipulations have focused on the negative outcomes of nutritional deficiency rather than on enhancements that could result from supplementation.

Morphological alterations

In these experiments, biocytin-filled CA1 pyramidal cells from prenatally choline-supplemented rats showed larger soma size and an increased number of primary and secondary basal dendritic branches. Such changes could clearly affect the physiological characteristics of the neurons. For example, the decrease in input resistance that we observed among neurons from prenatally choline-supplemented rats could result from the increased somatic size and thus the distribution of voltage-dependent conductances, as has been observed in certain CA3 neurons (Savic and Sciancalepore 2001). Likewise, dendrites are not only the primary sites of synaptic input but are also an important region where synaptic inputs are integrated before the initiation of action potentials. The Sholl analysis indicated that choline supplementation resulted in a greater density of basal dendrites, particularly in close proximity to the soma, suggesting the possibility that this morphological alteration could underlie changes in some of the firing patterns that we observed in this study.

CA1 pyramidal cells develop during embryonic days 16–20 (Bayer 1980), with a peak of neurogenesis around E18–E19. Thus in this study, prenatal choline supplementation was pro-

![Image of Figure 6]

**FIG. 6.** In response to brief depolarizing current injections, which elicited a train of 4 action potentials, the CA1 pyramidal cell from a prenatally choline-supplemented rat (B) showed a significant decrease in amplitude of slow afterhyperpolarizations (sAHPs) compared with that of the cell from a control rat (A). C: group data showing the mean ± SE amplitude of the AHP in cells from control (left, n = 33) and prenatally choline-supplemented (right, n = 35) rats. Calibration bars = 200 ms, 60 pA.
vided just prior to, and partly overlapping with, the neurogenesis of hippocampal principal cells. It is unknown how prenatal choline supplementation could alter the intrinsic morphology of CA1 pyramidal cells, but the results from other investigators indicated that, as a selective agonist, choline could exert its neurotrophic effects via activation of \(\alpha-7\) nicotinic acetylcholine receptors that are expressed in CA1 pyramidal cells (Albuquerque et al. 1997). This raises the interesting possibility that prenatal choline supplementation may contribute directly to the embryonic development of cholinergic system in the hippocampus, thus resulting in advanced maturity and synaptic function. Alternatively, choline also serves as a critical membrane ingredient, and additional choline availability could enhance membrane formation at a critical time, separate from any specific cholinergic action.

Interestingly, previous studies (Loy et al. 1991; Williams et al. 1998) showed that neuronal somata are larger in basal forebrain cholinergic neurons in rats that received prenatally choline supplementation and suggested that the neurotrophic effects of choline were restricted to the basal forebrain and not widespread. However, the present data indicate that choline may have more widespread effects on the brain.

**Alteration of intrinsic membrane properties**

CA1 pyramidal cells from prenatally choline-supplemented rats showed some alterations in their intrinsic membrane properties compared with those recorded from control rats. The difference in SFA between CA1 pyramidal cells recorded from control and prenatally choline-supplemented rats may be due to the reduced amplitude of the sAHP that we observed. The \(Ca^{2+}\)-activated \(K^+\) conductance, underlying the sAHP, is responsible for SFA in many types of neurons (Faber and Sah 2002; Johnston et al. 1994), and inactivation of this conductance leads to a reduction in SFA (Sah 1996). Thus it is possible that prenatal choline supplementation could result in an alteration of \(K^+\) conductances.

In addition, the sAHP and its underlying current, a calcium-dependent-potassium conductance, are subject to modulation by neurotransmitters including acetylcholine (Krause and Pedarzani 2000; Sah 1996; Storm 1990). Acetylcholine has been shown to reduce the amplitude of sAHPs in hippocampal principal cells through muscarinic mechanisms (Madison et al. 1987), and choline itself has been shown to excite cortical neurons by affecting membrane conductance (Knjivjevic and Reinhardt 1979). In a recent report, choline was found to exert...
its acute action on spontaneous action potentials of neurons in the hypothalamic tuberomammillary nucleus via α-7 nicotinic acetylcholine receptors (Uteshev et al. 2003). Thus studying the interaction between cholinergic mechanisms and ionic conductances, such as K⁺ channel regulation in prenatally choline-supplemented animals, may offer some clues about how dietary choline supplementation alters neuronal excitability in the hippocampus.

We also found an enhancement of the ADP in neurons from prenatally choline-supplemented rats. A variety of mammalian central neurons exhibit an ADP following either a single spike or a burst of action potentials. Although the exact physiological function of the ADP is unclear, the ADP could act to increase neuronal excitability. For example, an excitatory ADP observed in a subpopulation of hippocampal CA1 interneurons has been postulated to improve signal-to-noise ratios. Also, synaptic inputs sufficient to evoke an ADP could result in generation of action potentials in CA1 interneurons (McQuiston and Madison 1999). Therefore the ADP could play a key role in determining neuronal excitability. Although this has not been determined in hippocampal principal neurons, the present data suggest such a role.

Although some of the electrophysiological changes that we observed in CA1 pyramidal cells from prenatally choline-supplemented rats are rather small, such subtle shifts in cellular function may be very important for the function of those neurons and the local circuits in which they function over time. Such changes measured postnatally suggest that during cyto genesis prenatal choline affects the development and function of CA1 cells in subtle ways that are consistent with functional physiological changes. That such alterations can have marked effects on hippocampal function is illustrated by some studies of prenatal drug effects. For example, prenatal cocaine exposure has been shown to reduce AHPs and SFA in CA1 pyramidal cells from 20-day-old rats in the absence of effects on resting membrane potentials, action potential amplitude and duration, or membrane time constant (Baraban and Schwartzkroin 1997). These effects, although rather subtle from a neurophysiological standpoint, clearly suggested neuronal hyperexcitability within hippocampal circuits. Moreover, although we do not know if the treatment-associated changes we report in this study could persist across the life span of the animal, it has been suggested that increases in neuronal excitability like those we have observed could underlie a persistent bias toward neuronal excitability in the hippocampus and possibly promote memory storage in the hippocampal neurons in aged animals (Oh et al. 1999).

In addition, the enhancement of excitability and the change in neuronal structure that we observed in hippocampal cells are also consistent with the previously reported findings that prenatal choline availability leads to enhanced learning and memory processes (Meck et al. 1989). Thus the hippocampal changes reported here are possible mechanisms for explaining those behavioral effects of choline and may be related to the elevation of hippocampal NGF that has recently reported among prenatally choline-supplemented rats (Sandstrom et al. 2002). However, it should be noted that a widespread increase in principal cell excitability, without a concomitant increase in inhibition could be problematic. Such a bias toward excitation could lead to epileptogenesis or lowered seizure thresholds. However, prenatally choline-supplemented animals have proven to be more resistant to hippocampal excitotoxicity in a seizure model (Yang et al. 2002) and to cingulate gyrus neurotoxicity resulting from NMDA antagonists (Guo-Ross et al. 2002). Thus at present, there is no direct evidence for a propensity toward CNS excitotoxicity among prenatally choline-supplemented animals.

**Physiological significance of prenatal choline supplementation**

Since prenatal choline supplementation in rats increases learning ability, enhances synaptic function, and offers protection from neurotoxicity, should humans increase their intake of choline? Recently the Food and Nutrition Board of the Institute of Medicine classified choline as an essential nutrient and established recommended levels of choline intake for men and women, including elevated intake recommendations for pregnant and lactating women (see Blusztaijn 1998). In addition the Food and Drug Administration has recently approved labeling that lists whether a food is a good source of choline. The major sources of choline in the human diet are beef or chicken liver, eggs, wheat bran, fish, and beef. As some of these foods have become less popular because of their cholesterol content, perhaps some consideration should be given to whether humans should modify their diets to be certain they meet the recommended levels, particularly during the critical prenatal period.

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**References**


