Prenatal choline supplementation alters hippocampal N-methyl-d-aspartate receptor-mediated neurotransmission in adult rats

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Abstract

Manipulation of dietary choline levels in pregnant rats has been shown to result in enduring alterations in memory and hippocampal function of the offspring, but the mechanisms underlying these effects remain unclear. Hippocampal slices were prepared from adult rats that were offspring of dams fed control, choline supplemented, or choline deficient diets on days 12–17 of gestation. N-methyl-d-aspartate (NMDA) receptor-mediated population excitatory postsynaptic potentials (pEPSPs) were pharmacologically isolated and evoked using electrical stimulus pulses applied to s.radiatum of area CA1. Evoked NMDA receptor-mediated pEPSPs were enhanced in slices from prenatally choline supplemented relative to controls in both male and female rats. The greatest differences occurred at the low end of the input-output curve, among responses that were less than 60% of maximal. These results are discussed in the context of previous behavioral and electrophysiological studies.

Keywords: Prenatal; Choline; Hippocampus; N-methyl-d-aspartate; Rat; Development

Choline is an essential nutrient for which dietary guidelines have recently been established [1]. The guidelines for pregnant and lactating women were recommended in part on the basis of recent research that has shown a beneficial effect of prenatal choline supplementation on subsequent memory [3–8,11] and hippocampal function [2,10] in animal models.

The effects on hippocampal function are striking, and generally suggest an increase of excitatory synaptic function after prenatal choline supplementation and a decrease after prenatal choline deficiency. For example, we have shown that the stimulus intensity threshold for induction of N-methyl-d-aspartate (NMDA) receptor-dependent long-term potentiation (LTP) in hippocampal area CA1 is decreased in adult offspring after prenatal choline supplementation and increased after prenatal choline deficiency [2,10]. However, there is also some evidence that prenatal choline deficiency may increase certain aspects of hippocampal function. Meck and Williams [8] have shown that on certain behavioral tasks, prenatally choline deficient animals perform better than controls, at a level comparable to that of prenatally choline supplemented animals. In addition, we have shown a trend (though not statistically significant) toward enhancement of excitatory responsiveness in area CA1 of hippocampal slices prepared from prenatally choline deficient animals after transient exposure of the slices to Carbachol [9]. These studies raise the question of whether prenatal choline supplementation and deficiency may produce comparable effects on hippocampal function under certain circumstances.

The outcomes of our earlier LTP studies suggest that NMDA receptor-mediated activity in the hippocampus would be increased after prenatal choline supplementation and decreased after prenatal deficiency. The present study was designed to test that hypothesis.

Pregnant Sprague–Dawley CD strain albino rats were obtained from Charles River (Kingston, NY) at day 9 of gestation (E9), and housed individually in clear polycarbonate cages (27.9 × 27.9 × 17.8 cm3) on a 12-h light–dark cycle. Food and water were provided ad libitum. The dams were fed purified Dyets formula AIN-76A diet (Dyets Inc., Bethlehem, PA). Previous studies have shown that prenatal days 12–17 represent a critical period during...
which manipulation of dietary choline results in both behavioral [3–10] and electrophysiological changes in offspring. Therefore, dietary manipulation was carried out from day 12–17 of gestation. Control rats received an AIN-76A diet containing 7.9 mmol/kg choline chloride and water sweetened with 50 mM saccharine, resulting in an average daily choline intake of 1.3 mmol/kg per day. Dams in the choline supplemented group received AIN-76A diet, containing 7.9 mmol/kg choline chloride, and water, containing 25 mM choline chloride, sweetened with 50 mM saccharine. This resulted in an average daily choline intake of 4.6 mmol/kg per day (saccharine was used to neutralize the bitter taste of choline in the diet to equalize the water intake among dams in the treatment groups. Dams in the choline deficient group received an AIN-76A diet without added choline (0.0 mmol/kg per day) and water with 50 mM saccharine. These levels are consistent with behavioral and electrophysiological changes among offspring in previous studies [3–10].

After day 17 of gestation, all animals were fed normal AIN-76A diet containing 7.9 mmol/kg choline chloride and saccharine-free water. At birth the pups were cross-fostered to an untreated foster dam, and were weaned at postnatal day 24 (P24). At P30 they were housed two to a cage and provided with the standard, (i.e. with normal choline level) diet AIN-76A and water ad libitum.

Two separate experiments were run, one using male animals and the other using overectomized females. The experiment with males used nine offspring from control dams and nine from choline supplemented dams. The experiment with females used 12 offspring from control dams, 11 from choline supplemented dams, and ten from choline deficient dams.

In all instances the investigators were blind to the treatment condition of the animals during the electrophysiological experiments. The animals were no more than 4 months old when sacrificed. On each test day one rat was anesthetized with Halothane and decapitated. The brain was quickly removed and placed in ice-cold, oxygenated artificial cerebrospinal fluid (ACSF: containing (in mM) NaCl, 124; KCl, 3.5; NaH2PO4, 1.25; NaHCO3, 26; CaCl2, 3; MgSO4, 0.9; dextrose, 10; and continually oxygenated with 95% O2: 5% CO2 to maintain pH = 7.4). The brain was divided along the midline and 500 μm coronal slices (whole hemispheres containing the hippocampal formation) were cut using a Vibratome. The slices were allowed to equilibrate in ACSF for at least 2 h, and were then transferred to a submersion chamber that was maintained at room temperature and exposed to a mixture of 95% O2, 5% CO2 gas.

Recordings were made from stratum radiatum of area CA1 using a glass micropipette filled with 1 M NaCl (5–10 MΩ). A unipolar Tungsten wire electrode was used to stimulate afferent fibers in the stratum radiatum of CA1. The recording electrode was placed below stratum pyramidale and lowered until a clear population excitatory postsynaptic response (pEPSP) was reliably evoked. Test stimuli were single, square-wave pulses, 0.1 m in duration. Evoked field potentials were amplified ×10, filtered at 0–5 kHz, digitized at 10 kHz (16-bit) using a laboratory computer system, and stored on a hard disk for offline analysis. Prior to drug exposure an I/O curve was generated from each slice using stimuli of 100, 200, 400, 600 and 800 μA, presented at 8-s intervals. Slices were only used if it was possible to elicit a pEPSP of at least 0.5 mV with the 800 μA stimulus intensity. If five successive slices from a given animal failed to produce population spikes of this amplitude, tissue from that animal was not used.

After the completion of the I/O curve, a mixture of 10 μM of 6,7-dinitroquinoxaline-2,3(1H,4H)-dione (DNQX) and 10 μM picrotoxin (PTX) diluted in normal ACSF, was bath-applied to the slice for 40 min. During this period another I/O curve was generated. Following this treatment, 25 μM d,l-2-amino-5-phosphonovaleric acid (APV) was added to the bath to determine if the pEPSPs recorded in DNQX/PTX were NMDA receptor-dependent. Single stimulus pulses were used to generate NMDA receptor-mediated pEPSPs in slices from male rats. In the experiment with females, twin pulses (10 ms interstimulus interval) were used. All drugs used were obtained from Sigma.

Several measurements were taken from each of the field potential recordings. The amplitude of the pEPSP was measured in millivolts (mV) from the point of greatest positivity just prior to the onset of the negative-going pEPSP, to the point of greatest negativity within the deflection. The slope of the population pEPSP was measured in mV/ms from the initiation of the negative deflection to the maximal negativity in the waveform. In order for a slice to be considered viable for analysis, it had to produce an NMDA receptor-mediated pEPSP of at least 0.25 mV. Each pEPSP amplitude and slope measurement was expressed as a percentage of the maximal response for that slice.

Fewer hippocampal slices from the prenatally choline deficient group produced viable NMDA receptor-mediated pEPSPs than did those from either the control or prenatally choline supplemented groups (χ² = 4.46; P < 0.05). Therefore slices from the animals in the prenatally choline deficient group were not included in subsequent analyses.

Stable and consistent I/O curves were generated for NMDA mediated pEPSP amplitudes across a range of stimulus intensities from 100 to 800 μA. Fig. 1 shows the I/O curves for slices from control and prenatally choline supplemented animals. There was an overall significant effect of prenatal treatment (F(2,9) = 8.91; P = 0.007) and a treatment by stimulus intensity interaction (F(5,23) = 5.08; P = 0.003), indicating that the treatment effect varied across the I/O curve. Post-hoc comparisons indicated that the percent maximal responsiveness was higher than controls in slices from prenatally supplemented animals at stimulus intensities of both 200 and 400 μA. In addition, responsiveness of slices from prenatally choline supplemented animals was nearly significantly elevated relative to controls at 600 μA (P = 0.051). It is important to note that the greatest differences occurred at the low end of the
I/O curve among responses that were less than 60% of maximal.

Similar findings were observed when pEPSP slopes were analyzed (see Fig. 2). Again, there was an overall effect of prenatal treatment ($F_{2,13} = 5.19; P = 0.02$), a treatment by stimulus intensity interaction ($F_{2,52} = 5.57; P = 0.008$), and the enhanced responsiveness among slices from prenatally choline supplemented rats was observed at the lower stimulus intensities on the I/O curve (100, 200 and 400 μA).

As in the study of female rats, prenatal choline supplementation produced a greater responsiveness of pEPSP slope at low stimulus intensities on the I/O curve (Fig. 3). There was a significant effect of stimulus intensity ($F_{2,13} = 8.06; P = 0.007$), and a significant treatment by stimulus intensity interaction ($F_{2,13} = 4.33; P = 0.041$).

Post-hoc comparisons indicated that while the trend was for pEPSP slopes to be higher in slices from prenatally choline supplemented animals, this only reached statistical significance at the 100μA stimulus intensity ($t_{(8)} = 2.40; P = 0.04$). In contrast to the effects in females, however, there was no significant effect of prenatal choline supplementation on the measures of pEPSP amplitude.

The main finding of this study is that supplementation of dietary choline availability during gestation enhances NMDA receptor-mediated pEPSPs in the hippocampus of the adult offspring. Among slices from prenatally choline supplemented animals, the percent maximal responsiveness of pEPSPs was enhanced relative to controls. This effect was most pronounced at low stimulus intensities.

It is noteworthy that significantly fewer slices from prenatally choline deficient rats yielded electrophysiologically viable NMDA pEPSPs than did those from the control or choline supplemented groups. Given that there were no differences between the basal, (i.e. non-NMDA receptor-mediated) pEPSPs between the treatment groups, it may be that prenatal choline deficiency selectively compromises the subsequent functioning of the hippocampal NMDA neurotransmission. However, a formal test of this hypothesis will require further studies.

These results are consistent with our previous studies of LTP in hippocampal slices from prenatally choline supplemented rats [2,10]. In those studies we found that prenatal choline supplementation decreased the threshold for LTP induction in area CA1. The present findings suggest that the lowering of LTP threshold among prenatally supplemented animals could be the result of an enhancement of NMDA receptor-mediated neurotransmission in the Schaffer collateral-commissural/CA1 circuit. Although there are additional possible mechanisms underlying the enhancement of hippocampal excitatory responsiveness by prenatal choline supplementation, the present data show, for the first
time, that prenatal choline availability alters NMDA receptor-mediated function in adulthood. To our knowledge this is the first instance in which NMDA function has been altered by a dietary manipulation of any kind. That these data show a presumably permanent alteration after prenatal dietary manipulation is particularly interesting given that both LTP [2,10] and learning [7] are permanently altered after prenatal choline manipulation.

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