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Effect of Maternal Dietary Restriction on Serotonin and Catecholamine Levels of the Developing Rat Brain

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어미쥐의 식이섭취제한이 이유전 새끼쥐 뇌의 Seretonin과 Catecholamine 수준에 미치는 영향

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□ 국 문 요 약 □

분만전후 Sprague Dawley 암컷 쥐에서 양적으로 식이를 제한하여 임의로 먹인 Control group 의 일일평균섭취량이 1/2의 시중사료를 식이제한 group에게 주었다. Control group은 실험기간 동안 임의로 먹게 두었고, Deficient I group은 임신중에는 임의로 먹였고 분만후부터 이유시까지는 1일 20g의 사료를 주었다. Deficient II group은 임신 15일부터 분만시까지 1일 15g의 사료를 주었고 분만후부터 이유시까지는 1일 20g의 사료를 주었다.

각 group에서 새끼쥐가 출생한 날을 0일로 해서, 0, 3, 7, 10, 14, 21일에 새끼쥐의 serotonin, catecholamine 및 tryosine의 함량을 측정했다.

새끼쥐 뇌의serotonin함량은 control group에 비해 식이제한 group에서 출생 7일부터 유의적으로 낮아졌으며, 두 식이제한 group사이에서는 출생 14일과 21일에 유의적인 차를 보였다.

뇌에서 norepinephrine과 dopamine같은 catecholamine은 Control group보다 식이제한 group에서 유의적으로 낮았지만, 두 식이제한 group사이에서는 유의적 차를 보이지 않았다.

Catecholamine의 전구체인 tyrosine은 식이제한 group의 뇌에서보다 Control group에서 출생 3일에 유의적으로 낮았으며, 출생 14일에는 Deficient I group의 tyrosine 함량이 Control group과 Deficient II group의 뇌에서 보다 유의적으로 낮았다.

임신 마지막 주와 수유시의 어미쥐의 식이제한은 새끼쥐 뇌에서 정상적인 catecholamine합성을 지연시켜 주는 것으로 추측되며, 만일 식이제한을 시키는 기간을 이유 후로 연장시켜 주면, 뇌에서 유의적으로 낮은 catecholamine의 함량이 정상으로 회복될 수 있으리라 기대된다.

본 연구결과를 통하여 볼 때, 분만전후의 어미쥐에게 영양부족을 시키면, 새끼쥐 뇌성장발육과 조성이 정상적으로 될 수 없음을 알 수 있다.

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ABSTRACT

A quantitative restriction of maternal diet was given to the Sprague Dawley rats during the third week of gestation and during the lactation. Dams of deficient groups were fed 20g of a stock diet a day and compared with the control group fed ad libitum throughout the experimental period. Dietary restriction started from birth in deficient 1 group and from the 14th day of gestation in deficient 2 group. Brain serotonin, norepinephrine, dopamine, and tyrosine of offsprings were determined at several intervals. Brain serotonin of offsprings of deficient groups 1 and 2 were significantly lower than the control and significant differences between the deficient groups were noticed. Brain catecholamines and tyrosine of offsprings of deficient groups were significantly lower than the control and there was no difference between the deficient groups. Maternal dietary restriction during the gestation and the lactation may have retarded the synthesis of serotonin and catecholamines in the brain.

INTRODUCTION

Early undernutrition changes in biochemical composition and functions of the brain of humans (1) and rats (2–4). The cellular and structural integrity of the brain is critical to the proper brain function and brain cellularity and connectivity (function) may be most vulnerable to a dietary restriction during a "critical period of development", when brain growth is most rapid (1). However, the appropriate number of brain cells and correct connectivities among them are not always enough to guarantee normal brain function. Brain neurons must also be able to synthesize and release neurotransmitter molecules. The function of neurotransmitter is to relay electrical impulses chemically among the neurons that do not make direct membrane contact with each other. This chemical function of neurons has been suspected to be susceptible to diet and malnutrition (5) as all of the known or suspected neurotransmitters are themselves constituents of the diet (amino acid such as glycine, aspartate, glutamate), or relatively simple metabolic products of dietary constituents (eg. serotonin from tryptophan, norepinephrine and dopamine from phenylalanine or tyrosine, GABA from glutamate, histamine from histidine, and acetylcholine from choline).

The catecholamines, norepinephrine and dopamine and the indolamine, serotonin, are the brain monoamines on which interest has been focused. Norepinephrine is synthesized from the amino acid tyrosine through the intermediates 3,4–dihydroxyphenylalanine (dopa) and dopamine: it is stored in introneuronal granules. Serotonin is synthesized from the precursor amino acid tryptophan by decarboxylation and, like norepinephrine, may exist in the neuron as a bound and a free form. Recently it is recognized that rapid and specific changes in brain constituents including a neurotransmitter and its amino acid precursor, occur after each meals. It was suggested that effect of protein malnutrition be either to delay the formation of neurotransmitter synapses, or to inhibit the synthesis or storage of the amine (6). Therefore, the concentration of these neurotransmitter in brain may be decreased by undernutrition. Wurtman and Fernstrom (7) have shown that the brain of rats whose mother were fed 8% protein diet during the pregnancy and the lactation contained significantly less norepinephrine and dopamine than the brains of offspring from well–nourished dams. Smart (8) reported that brain norepinephrine and dopamine contents in 24–day old deprived rats were decreased and tyrosine hydroxylase activity increased. However, Sobotka et al (9) reported that there were no changes in catecholamine levels in 22–day old deprived rats. Thus, it is still not clear whether the change in brain catecholamine level resulted from perinatal undernutrition is permanant, even after a prolonged period of normal feeding.

The purpose of this investigation was to determine the effect of a dietary restriction during the third week of gestation and lactation on brain serotonin, norepinephrine, dopamine, and tyrosine contents.

MATERIALS AND METHODS

Virgin female rats of Sprague Dawley strain supplied by animal breeding laboratory of Seoul National University were used in this experiment. Stock diet was obtained from Jeil Fodder Co., Seoul, Korea. The scheme of experimental design was illustrated in the previous paper (10). Control group fed a stock diet ad libitum during the gestation and lactation : deficient 1 (Def 1) fed a restricted diet (20g/day) from both ; deficient 2(Def 2) from the 15th day of gestation. At the age of 0, 3, 7, 10, 14, and 21 days of postnatal period, offsprings were taken randomly from each group and the brain were analyzed for serotonin (11), catecholamines(11), and tyrosine (12). Most of the chemicals used in this experiment were obtained from Sigma (USA) and the Farrand Spectrofluorometer was used. Experimental data from the control and the deficient groups were analyzed by Student's t – test.

RESULTS AND DISCUSSION

Previous experiment (10) by this lab showed that the body and brain weights of deficient pups were significantly lower than the control, but the ratios of brain weight to body weight in deficient groups were higher than the control. Significant differences of ratios between deficient groups were noticed at weaning. Brain DNA, RNA, and total protein of offsprings of deficient groups were significantly lower than the control, but RNA/DNA, brain weight/DNA and total protein/DNA showed that cell number were more affected than the cell size by the maternal dietary restriction during the 3rd week of gestation and lactation. Between the deficient groups, there was a significant difference in brain DNA and RNA, but no significant difference in total brain protein.

Deficient pups were either stawed to death or camilalized by their dams so they could not survive beyond the weaning.

Serotonin content in brain

Brain serotonin contents are shown in Fig. 1 As early as 7th day of postnatal period, the brain serotonin contents of deficient groups were significantly lower than the control. Even between the deficient groups, there was a significant difference in brain serotonin content at 14th and 21st day.

The substrates of neuronal intercommunications are neurotransmitters : these compounds are released by depolarizing neurons, and have the ability to de polarize other neurons. This study was concerned with the change in the ability of neurons to synthesize and release normal amounts of neurotransmitters. It is believed that the diet influences the formation of these transmitters by making more or less of each of their respective precursors available. This is based on the fact that the enzyme catalyzing the rate-determining step in the formation of each transmitter is relatively unsaturated with substrate Thus, increase or decrease in the amount of tryptophan in brain rapidly stimulate or reduce the rate of serotonin formation.

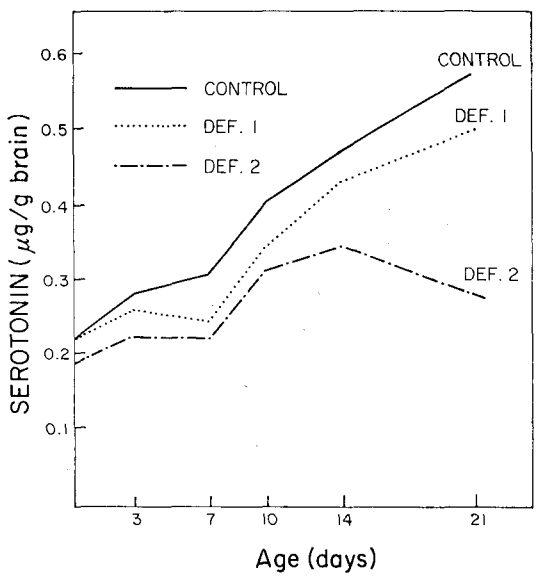

Fig. 1. Effect of maternal dietary restriction on brain serotonin of offsprings.

Table 1. Effect of matetary restriction on brain tyrosine of offsprings (mg/ whole brain)

Age (days)	Control	Deficient 1	Deficient 2
0	0.209±0.065 [a] (17)[b]	0.209±0.065 (17)	0. 172±0. 062 (17)
3	0.100±0.041[++] (7)	0.310±0.040 (10)	0. 378±0. 059 (11)
7	0.880±0.062 (6)	0.854±0.204 (6)	0. 678±0. 070[**] (7)
10	1.300±0.415 (4)	0.609±0.133 (4)	0. 764±0. 087 (5)
14	1.281±0.089 (5)	0.194±0.099[**] (5)	0. 996±0. 255 (5)
21	1.408±0.206 (3)	0.760±0.230 (7)	1. 253±0. 134 (4)

a Mean±S.D.
b Number of animals sacrificed.
** P< 0.01 : significantly different from the control.
++ P< 0.01 : significantly different from the deficient.

The amount of tryptophan available to the brain might control the serotonin synthesis was suggested by the four lines of evidence (7): 1) The existence in rats and mice of a diurnal rhythm in brain content; 2) The likelihood that tryptophan hydroxylase an enzyme that catalizes the initial step in serotonin biosynthesis, might not normally be fully saturated in vivo; 3) Published evidence that very high dose of tryptophan and serotonin could increase brain serotonin; 4) Tryptophan deprivation by malnutrition lowered tryptophan and serotonin.

Fernstrom and Lytle (13) carried out the studies on the effect of corn malnutrition on brain serotonin. The chronic ingestion of the corn diet reduces tryptophan intake via the diet. This lowering of tryptophan intake ultimately leads to a depression in serum and tissue pools of the amino acid, and notably a reduction in brain tryptophan concentrations. A predominant effect of the reduced size of the brain tryptophan pool is probably to lower the degree of substrate saturation of tryptophan hydroxylase in those neurons containing the enzyme. The fall in substrate saturation of tryptophan hydroxylase slows the rate of conversion of the amino acid to serotonin, which ultimately is expressed as a decline in brain serotonin and 5-hydroxyindoleacetic acid concentration.

The integrity of many types of behavior (pain sensitivity, locomotor activity, sleep, and sex) depends in part on the normal function of serotonin-containing neurons in the brain (14). Therefore, abnormal content of serotonin by malnutrition can cause the abnormal behavior patterns.

Catecholamines and tyrosine contents in brain

Norepinephrine content in the whole brain of offsprings are shown in Fig. 2. In Def 1 and 2 groups

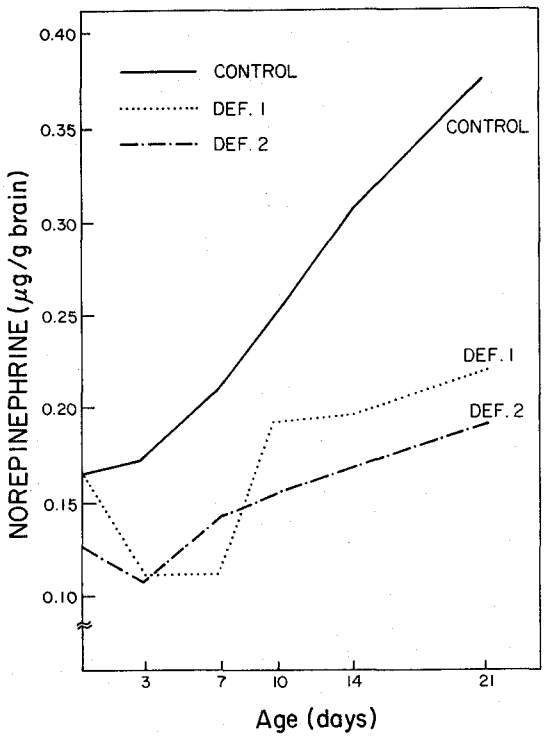

Fig. 2. Effect of maternal dietary restriction on brain norepinephrine of offsprings.

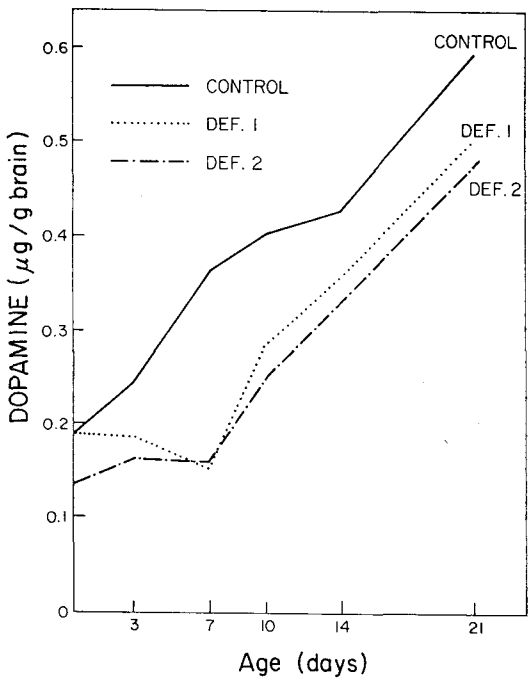

Fig. 3. Effect of maternal dietary restriction on brain dopamine of offsprings.

the norepinephrine contents in brain of offsprings were significantly lower than the control. There was no significant difference in norepinephrine between the deficient groups.

Dopamine content in the whole brain of offsprings are depicted in Fig. 3. In the deficient groups, brain dopamine contents of offsprings were significantly lower than the control. Between the Def 1 and 2, there was no significant difference in brain dopamine content.

Brain tyrosine contents are shown in Table 1. In Def 1 and 2, tyrosine content was significantly lower than the control.

Shoemaker and Wurtman (6) have reported that the most of the dopamine deficit by prenatal undernutrition was localized within the neurons of basal ganglia. These decreases in brain apparently do not reflect decrease in the activity of the principal enzymes of catecholamine biosynthesis. No dopa was detected in the brains of malnourished animals. Brain dopamine and norepinephrine were not significantly elevated and tyrosine, the precursor for norepinephrine and dopamine was concentrated in the brains of undernourished rats at the expense of the rest of the body. Brain tyrosine concentrations were identical in the well-nourished and the undernourished rats. They also reported that the increase in brain tyrosine hydroxylase activity, the selective concentration of tyrosine within the brain, and the decrease in the turnover rate of brain norepinephrine all suggest that brain catecholamines are conserved when nutrient supply limits their synthesis in the developing rats.

Sereni et al (15) have shown that undernutrition during the suckling period significantly depressed the concentration of catecholamine and returned to normal by prolonging undernutrition until 35 days of age. Therefore, it seemed that maternal dietary restriction during the gestation and the lactation retarded the synthesis of catecholamin. If the period of undernutrition is prolonged, it is expected that significantly low concentration of catecholamine is recovered to normal.

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