EFFECT OF UNDERNUTRITION AND SUBSEQUENT REHABILITATION ON BRAIN 5-HT (SEROTONIN) PROFILE OF DEVELOPING NEWBORN RATS

K P SINGH, A K SANYAL

ABSTRACT

For littermate comparison, undernutrition in half of the pups of a litter was induced by 12h maternal deprivation from day 5 to day 18 postnatal. Subsequently, undernourished suckling rats were rehabilitated. Brain 5-HT concentrations and its turnover rate were measured on different days of age up to the period of 3 months. Brain 5-HT concentration was approximately half of the adult level at birth and increased progressively, except with a steep fall on day 18 postnatal, to the adult level by day 25 postnatal and thereafter it did not vary much on different days of age. Undernourished newborn rats showed significantly low level of brain 5-HT on day 9 and significantly higher concentrations on day 12 and 18 postnatal, whereas, rehabilitated rats showed comparable brain 5-HT concentrations with that of their nourished littermates. It is concluded that undernutrition had affected the brain 5-HT concentration and its turnover rate. Brain 5-HT concentration was low during early phase and was more during late phase of undernutrition. Rehabilitation of undernourished rats restored the brain 5-HT concentration similar to that of their nourished littermates.

KEY WORDS: Nutrition, Serotonin, Animal experiment.

INTRODUCTION

The existing literature on the effect of undernutrition on brain 5-HT (serotonin) steady state levels and its turn over rate is full of controversy. Some workers have reported increased levels while others have reported a decreased levels or no change in its concentrations. Such discrepancy in brain 5-HT levels of undernourished newborn rats could be either due to the variations in the duration of nutrition insult imposed or to the different methods used for inducing undernutrition by these workers. In these studies, interlitter nutritional status of both nourished and undernourished groups varied widely as the lactating mothers rearing the different litters might have demonstrated differential lactating capacity or there was an intralitter mutual competition for the teats in which relatively stronger pups commonly displaced the weaker ones. The above premise, therefore, prompted us to study the effect of undernutrition in the pups of a same litter in which 50% of the pups of a litter were undernourished by 12h of maternal deprivation.

MATERIAL AND METHODS

Induction of malnutrition in new born rats

Undernutrition in developing newborn rats was induced by 12h (from 9.00 pm to 9.00 am next morning) maternal deprivation of 50% of the pups of a litter according to the method of Mishra et al as described earlier. Thereafter, undernourished pups were rehabilitated on normal feeding similar to that of nourished littermates.

Estimation of brain serotonin and rate of accumulation

Brain 5-HT concentration (whole brain minus cerebellum) was estimated according to the method described by Curzon et al. In short, new born rats were decapitated between 9:30-10:30 A.M. Brain was quickly dissected out and put in 6-8 ml ice cooled acid-butanol. After removing both cerebelli, the brain was weighed and homogenized for 3 min in a Potter Elvehjem homogenizer with 10 volumes of ice cooled acid-butanol. The content was centrifuged at 3000 rpm for 5 min and the supernatant (4 ml) was pipetted into glass stoppered tube containing 8 ml of ice cooled n-heptane and 0.6 ml of 0.1 N. HCl (containing L-cysteine 1mg/ml). The mixture was shaken for 1-4 interlitter nutritional status of both nourished littermates.

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The mean 5-HT levels of whole brain (excluding cerebellum) in between 12-16h of the birth, was 557 ± 32 ng/g of fresh tissue. On the second day of life its level decreased significantly (453 ± 22 ng/g). Subsequent estimation from day 5 to day 14 postnatal demonstrated a gradual increase in brain 5-HT levels. Surprisingly, there was a steep fall in brain 5-HT levels (445 ± 20 ng/g) on day 18 postnatal. It rose again on day 25 postnatal (904 ± 30 ng/g) followed by a slight fall on day 36 (Table 1). Thereafter it rose very slowly to attain brain 5-HT levels similar to or slightly
higher than that observed on day 25 postnatal. The mean 5-HT level estimated on day 90 postnatal was the order of 944 ± 23 ng/g of fresh brain tissue.

The developmental profile of brain 5-HT in undernourished developing newborn rats was qualitatively identical but quantitatively distinctly different to that of their respective nourished littersmates. Initially, on day 9 postnatal, undernourished newborn rats showed significantly lower brain 5-HT concentration (559 ± 21 ng/g) as compared to that of nourished littersmates (626 ± 20 ng/g). Afterwards, brain 5-HT concentrations in undernourished animals increased at a much faster rate, which were significantly higher on day 12 and day 18 postnatal. Undernourished newborn rats rehabilitated for one week or more showed comparable brain 5-HT levels to that of their respective nourished littersmates (Table 1).

**Effect of undernutrition on the rate of accumulation of brain 5-HT**

Accumulation of brain 5-HT was significantly low on day 9 (123.1 ± 13.5 ng/g/h as compared to 162.2 ± 41.7 ng/g/h), practically same on day 10 (152.5 ± 22.4 ng/g/h and 151.0 ± 22.7 ng/g/h) and significantly higher on day 18 postnatal (392.2 ± 16.2 ng/g/h as compared to 285.6 ± 23.9 ng/g/h) in undernourished newborn rats when compared with nourished littersmates (Table 2).

**DISCUSSION**

Available literature on the normal developmental profile of brain 5-HT⁹ and the effect of undernutrition¹⁰ on its steady state level and turn over rate in developing newborn rats is equivocal. In spite of some disagreement, there is a general consensus that brain 5-HT level is low at birth and gradually increases to attain the adult levels by 3-5 weeks of age.³⁵⁻¹⁰ In the present study, brain 5-HT levels when measured between 12-16 h after birth on day 1 was 557 ± 22 ng/g/h of fresh tissue. It's level decreased by 19.6% on day 2 and then progressively increased, except a steep fall on day 18 postnatal, to attain the adult levels by day 25 postnatal. Therefore, brain 5-HT levels remained 'more or less' steady till the last day of observation i.e. on day 90 postnatal. Higher levels of brain 5-HT immediately after birth has also been reported by Bourgoin et al.¹⁰ An increase in brain 5-HT level has been reported by Sanyal et al.¹¹ after 1-2 h of immobilization stress and by Vermes et al.¹² after ACTH and corticosterone administration. Holt et al.¹² have reported 3.5 fold increase in plasma corticosterone levels within 5h of birth and suggested that stress of parturition was responsible for higher rate of corticosterone production by rat’s adrenals. It is presumed, therefore, that the higher level of brain 5-HT observed in the present study within 16h of birth could be due to the release of glucocorticoids¹² associated with the stress of parturition, while, lower levels of 5-HT in brain observed on the second day of birth could be due to passing of the stress associated with the parturition. The observed low level of brain 5-HT at birth could be related to the poor development of serotonergic innervation in brain.²⁰ Progressive increase in brain 5-HT from day 2 onward to day 14 postnatal might be due to simultaneous development of serotonergic innervation and increase in tryptophan hydroxylase activity.¹⁴ Likewise, the steep fall in brain 5-HT levels observed on day 18 postnatal could be explained on the basis of its rapid catabolism since the rate of accumulation of brain 5-HT was maximum (285.6 ± 23.9 ng/g/h) on this day as compared to 9 days old new born developing rats (162.2 ± 11.7 ng/g/h) and matured adult rats (216.6 ± 13.4 ng/g/h) respectively, after inhibition of MAO by pargyline. Bourgoin et al.¹⁵ had also reported 80% higher MAO activity in 15 days old rats as compared to that of adult ones. The development of serotonergic innervation and tryptophan hydroxylase activity to its maximum levels¹⁴ and decreased MAO activity¹⁵ in matured rats could be the reason for the higher values of brain 5-HT observed in the present study in 25 days old rats. Afterwards, brain 5-HT levels of adult rats maintained on a balanced diet were 'more or less' steady when estimated on different days of age.

Likewise undernourished developing newborn rats showed wide variations in brain 5-HT concentration when estimated on different days of their life. It's level decreased significantly on day 9 postnatal i.e. just after 3 days of the initiation of the undernourishment (559 ± 21 ng/g as compared to 626 ± 20 ng/g in nourished littersmates), became more or less same on day 10 and 11 and were significantly higher on day 12 (813 ± 25 ng/g as compared to 648 ± 35 ng/g in nourished littersmates) and on day 18 (561 ± 29 ng/g as compared to 445 ± 20 ng/g in nourished littersmates) but not on day 14. When 18 days old undernourished developing rats were rehabilitated on normal feeding, they showed comparable brain 5-HT

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**Table 1:** Effect of maternal deprivation and subsequent nutritional rehabilitation on developmental profile of brain steady state Serotonin (5-HT) concentration of developing newborn rats

<table>
<thead>
<tr>
<th>Age in days</th>
<th>Brain 5-HT concentration (ng/g of fresh tissue) Mean ± S.E.</th>
<th>Age in days</th>
<th>Brain 5-HT concentration (ng/g of fresh tissue) Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>557 ± 22 (3*)</td>
<td>25</td>
<td>904 ± 30 (4)</td>
</tr>
<tr>
<td>2</td>
<td>453 ± 22 (3*)</td>
<td>36</td>
<td>822 ± 37 (4)</td>
</tr>
<tr>
<td>5</td>
<td>621 ± 16 (7)</td>
<td>58</td>
<td>837 ± 29 (5)</td>
</tr>
<tr>
<td>9</td>
<td>626 ± 20 (5)</td>
<td>90</td>
<td>944 ± 23 (5)</td>
</tr>
<tr>
<td>10</td>
<td>643 ± 18 (4)</td>
<td>11</td>
<td>692 ± 26 (4)</td>
</tr>
<tr>
<td>12</td>
<td>684 ± 35 (5)</td>
<td>13</td>
<td>715 ± 25 (5)</td>
</tr>
<tr>
<td>14</td>
<td>692 ± 26 (4)</td>
<td>15</td>
<td>561 ± 29 (4)</td>
</tr>
<tr>
<td>18</td>
<td>445 ± 20 (5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

C control, MD maternally deprived, MDR maternally deprived – rehabilitated newborn rats. Figures in parentheses indicate number of rats used. * Brain of two new born rats were pooled for estimation of 5-HT. P values *< 0.5

**Table 2:** Effect of maternal deprivation on the rate of accumulation of brain Serotonin (5-HT) concentration of developing newborn rats after pargyline (75 mg/kg, i.p.) administration

<table>
<thead>
<tr>
<th>Age in days</th>
<th>Rate of accumulation of brain 5-HT concentration (ng/g/h) Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>162.2 ± 11.7 (11)</td>
</tr>
<tr>
<td>10</td>
<td>151.0 ± 22.7 (10)</td>
</tr>
<tr>
<td>18</td>
<td>285.0 ± 53.9 (9)</td>
</tr>
</tbody>
</table>

C control, MD maternally deprived newborn rats, Figures in parentheses indicate number of rats used, P values *< 0.5, † < 0.01
concentrations to that of their nourished littermates. If rate of accumulation of 5-HT is considered to be an index of the rate of synthesis, the rate of synthesis of 5-HT was found to be significantly less on day 9, equal on day 10 and significantly more on day 18 postnatal in undernourished newborn rats as compared to that of their nourished littermates, implying that serotonergic neuronal activity of undernourished brain of newborn developing rats was considerably less during the early phase of undernourishment but was significantly more during the established phase of undernutrition. On the other hand, rehabilitated undernourished developing rats showed comparable brain serotonergic neuronal activity with that of their respective nourished littermates.

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