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Effect of prenatal phenytoin administration on brain tryptophan metabolism of rat offspring during the preweaning period

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Serum 5-hydroxytryptamine (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) concentrations in control rat offspring increased progressively during the preweaning period reaching adult values by day 21. It has been shown that prenatal phenytoin administration (100 mg kg^{-1} orally, days 7–19 of pregnancy) increased serum tryptophan and brain tryptophan, 5-HT and 5-HIAA of rat offspring at 3 days of age but not at 4, 15 or 21 days of age. The effect of prenatal phenytoin administration on the offspring at 3 days of age was not observed when these pups were cross-fostered to control mothers at 2 days of age suggesting that the alteration in brain tryptophan metabolism during the development of tryptaminergic neurons in rat offspring, as a result of prenatal phenytoin administration is mediated through changes in lactation or nursing ability of the mothers. It is important that such non-specific factors are controlled when studying the effect of prenatally administered drugs on neonatal brain transmitter concentrations.

Clinical data indicate an incidence of mental subnormality that is higher than expected among children of epileptic mothers taking anticonvulsant drugs (including phenytoin) during pregnancy (Speidel & Meadow 1972; Hanson et al 1976). A delay in development of rat offspring treated prenatally with phenytoin has also been observed (Ata & Sullivan 1977). Numerous studies have demonstrated the functional role of 5-HT in the central nervous system and its relation to behaviour in animals (Smith et al 1976). The 5-HT containing nerve cells develop at a very early stage during ontogeny in the rat (Nelson et al 1975), and the brain concentration of 5-HT increases progressively with age during the preweaning period (Bourgoin et al 1974).

Phenytoin has been shown to increase brain 5-HT in the rat (Anderson et al 1962) and in the mouse (Jenner et al 1975; Chadwick et al 1978) after acute administration, and to increase brain 5-HT turnover in the rat (Green & Grahame-Smith 1975) and in man (Chadwick et al 1975) after chronic administration.

The object of this study was to investigate the effect of chronic phenytoin administration during pregnancy in the rat on brain tryptophan metabolism of the rat offspring during the preweaning period to see whether any permanent effect was produced by such treatment.

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METHODS

Adult male and virgin female Wistar rats (200–250g) were housed under conventional conditions using a 12 h light-dark cycle. Food (B.P. Nutrition rat and mouse No. 3 expanded diet) and water were freely available (unless specified).

Female rats were mated overnight using one male in each cage with 5 females. Next morning, the females were examined for the presence of spermatoza in the vagina. Rats with a positive smear were separated in pairs and divided into two groups and allowed free access to food and water from days 1–7 of pregnancy. Day 1 was designated as the day of finding spermatoza in the vaginal smear. The pregnant rats were treated daily from days 7–19 of pregnancy with phenytoin (Epanutin, Parke-Davis, 100 mg kg^{-1}) or an equivalent volume of water (5 ml kg^{-1}) orally by gavage. Since it was known that this dose of phenytoin would reduce food intake the control group was pair-fed with the treated group during the whole treatment period. After the last dose at day 19 of gestation, the rats were separated individually into plastic cages with wood shavings and paper tissues as nesting material, received food and water freely and were allowed to deliver spontaneously. The offspring were culled to 6–8 pups per litter within 24 h after parturition, when possible equal numbers from each sex were kept in each litter.

In one experiment, 2 or 3 pups from each litter were decapitated between 9–10 a.m. at 3, 4, 15 and 21

days of age (the day of parturition is designated as day one of age). The blood was collected from the trunk and used for the separation of serum, and the brain quickly removed and frozen at −20 °C.

In a second experiment, the offspring were culled to 8 and were fostered or cross-fostered within 24 h after parturition. The pups were either transferred to the nest of a mother from the same treatment group (fostered) or to that of a mother from the other treatment group (cross-fostered), i.e. none of the litters were reared by their own mothers. This procedure resulted in 4 groups, control pups reared by control mothers (Cc), control pups reared by treated mothers (Tc), treated pups reared by treated mothers (Tt) and treated pups reared by control mothers (Ct). Two pups (a male and a female) from each litter were killed 24 h after fostering or cross-fostering, i.e. at 3 days of age. The blood sample and brain were removed as above and the stomach was also dissected out and the stomach milk content was weighed for each pup.

The whole brain was used for the fluorimetric determination of brain tryptophan, 5-HT and 5-HIAA according to Curzon et al (1972). 0·1 ml of the serum was used for the fluorimetric determination of serum tryptophan and 5-HT using the same method. The results were analysed using Student's *t*-test, or using the method of multiple comparison with a control (Dunnett 1964).

RESULTS

Effect of prenatal phenytoin administration on brain tryptophan metabolism of rat offspring.

Two groups of 15 and 14 pregnant rats were used, the latter group being treated with phenytoin. The rats were weighed on days 1, 7, 10, 13 and 19 of pregnancy. The body weights increased from a mean of 225 g on day 1 to 255 g on day 7 when phenytoin treatment was started. The weights decreased to 245 g by day 10 and then progressively increased to 310 g by day 19. As a result of pair feeding the control group, the mean weights of the control and treated dams did not differ by more than 4 g from the means quoted above.

The results of the effect of prenatal phenytoin administration on the body weight, brain weight, serum tryptophan and 5-HT and brain tryptophan, 5-HT and 5-HIAA concentrations of the rat offspring during the preweaning period are shown in Table 1.

In general, the body and brain weights of the treated pups were slightly less than those of the controls but were only significantly so on day 3. The brain : body weight ratios however were similar throughout. In control pups the serum tryptophan concentrations showed no consistent change with age but the serum 5-HT concentrations were low up to day 4 but had reached adult values of around 1 μg ml^{-1} by day 15 of age. Similarly the brain 5-HT concentrations were low at days 3 and 4 but increased towards adult values by day 21. Except for day 3, the values in the treated pups were very similar to those in the controls. On day 3 however the serum tryptophan and brain tryptophan, 5-HT and 5-HIAA were all significantly higher in the treated pups than in the controls.

To see if the difference observed on day 3 was related to food deprivation during the immediate post-partum period a second experiment was carried out in an attempt to control for this.

Table 1. Effect of prenatal phenytoin treatment (100 mg kg^{-1} orally, days 7–19) on brain tryptophan metabolism of rat offspring during the preweaning period.

	Days of age							
	3		4		15		21	
Treatment group	Control	Phenytoin	Control	Phenytoin	Control	Phenytoin	Control	Phenytoin
(Pup No/litter No)	(14/6)	(13/5)	(12/6)	(10/4)	(11/6)	(7/3)	(9/3)	(8/3)
Body weight (g)	7·66 ± 0·47	6·85 ± 0·29	9·52 ± 0·36	8·48 ± 0·41	32·1 ± 2·34	27·83 ± 4·55	33·44 ± 1·54	38·4 ± 0·92
Brain weight (mg)	329·00 ± 8·3	304·00 ± 5·8*	395·00 ± 7·6	366·0 ± 15·8	1140·00 ± 35·7	1126·00 ± 66·4	1375·00 ± 34·6	1334·00 ± 16·5
Brain weight (g)/ Body weight (g)	0·044 ± 0·0017	0·045 ± 0·0017	0·042 ± 0·0010	0·043 ± 0·0005	0·036 ± 0·0023	0·042 ± 0·0058	0·041 ± 0·0009	0·035 ± 0·0012
Serum tryptophan (μg ml^{-1})	10·24 ± 1·78	23·04 ± 5·27*	11·19 ± 1·33	16·84 ± 5·34	23·12 ± 1·42	20·51 ± 3·31	14·89 ± 0·75	17·42 ± 1·70
Serum 5-HT (μg ml^{-1})	0·218 ± 0·021	0·245 ± 0·033	0·283 ± 0·011	0·320 ± 0·012	1·125 ± 0·114	1·040 ± 0·152	0·989 ± 0·094	0·813 ± 0·053
Brain tryptophan (ng/100 mg)	331·00 ± 50·6	815·00 ± 106·9**	389·00 ± 65·5	606·00 ± 196·2	527·00 ± 14·7	612·00 ± 73·1	388·0 ± 33·8	366·0 ± 4·5
Brain 5-HT (ng/100 mg)	27·4 ± 2·07	37·2 ± 1·64**	25·8 ± 1·20	29·4 ± 1·83	37·4 ± 1·13	36·0 ± 2·94	58·9 ± 1·97	55·0 ± 0·91
Brain 5-HIAA (ng/100 mg)	44·2 ± 4·37	82·7 ± 11·61**	60·2 ± 5·84	88·5 ± 18·13	88·3 ± 1·65	93·4 ± 10·2	100·7 ± 7·15	98·5 ± 4·81

Results are expressed as means of the means of the litters ± s.e. Results are compared against the corresponding control results by Student's *t*-test.

* $P < 0.05$ ** $P < 0.01$

Table 2. Effect of prenatal phenytoin treatment (100 mg kg^{-1} orally, days 7–19) and of fostering or cross-fostering on brain tryptophan metabolism of rat offspring at 3 days of age.

	Treatment group (Number of pups/Number of litters)			
	Cc (18/9)	Tc (12/6)	Tt (14/7)	Ct (12/6)
Body weight (g)	7·42 ± 0·27	6·35 ± 0·20*	5·78 ± 0·20**	6·61 ± 0·32
Brain weight (mg)	339·00 ± 8·19	311·00 ± 7·01*	305·00 ± 4·14**	312·00 ± 5·97*
Stomach content weight (mg)	338·00 ± 38·8	205·00 ± 24·2*	92·00 ± 18·6**	279·00 ± 32·7
Serum tryptophan ($\mu g\ ml^{-1}$)	9·14 ± 0·81	10·64 ± 1·39	18·41 ± 2·79**	7·23 ± 0·8
Serum 5-HT ($\mu g\ ml^{-1}$)	0·171 ± 0·004	0·190 ± 0·009	0·255 ± 0·024**	0·171 ± 0·013
Brain tryptophan (ng/100 mg)	405·00 ± 34·49	527·00 ± 95·8	1154·00 ± 245·9**	427·00 ± 88·6
Brain 5-HT (ng/100 mg)	31·6 ± 0·69	33·3 ± 2·33	45·5 ± 2·78**	31·8 ± 2·78
Brain 5-HIAA (ng/100mg)	69·2 ± 4·18	88·0 ± 9·57	119·6 ± 11·58**	72·1 ± 16·73

* $P < 0·05$ ** $P < 0·01$

Effect of prenatal phenytoin administration and of fostering or cross-fostering on the brain tryptophan metabolism of 3 days old rat offspring.

Two groups of 15 and 13 pregnant rats were used, the latter group being treated with phenytoin. The controls were pair-fed with treated rats from days 7–19 of pregnancy and the weights and weight gains were virtually identical with those observed in the first experiment above. One day after delivery the pups were culled and fostered or cross-fostered as described under Methods and resulted in the following numbers of litters in each group: control fostered (Cc) 9, control cross-fostered (Tc) 6, treated fostered (Tt) 7, and treated cross-fostered (Ct) 6.

On day 3 the pups were weighed and one male and one female pup from each litter killed for measurement of serum tryptophan and 5-HT, brain tryptophan, 5-HT and 5-HIAA and weight of milk in the stomach. From the analysis of variance of the data, there were no treatment related sex differences in any of the parameters measured so the results from males and females have been pooled and the results are shown in Table 2. The values in the treated (Tt) pups were similar to those obtained in the first experiment i.e. with reduction in body weight and elevation in tryptophan, 5-HT and 5-HIAA concentrations. However the concentrations in the cross-fostered treated (Ct) pups were not significantly different from the controls (Cc) although their body and brain weights were still reduced. The whole of this second experiment has been repeated with virtually identical results.

DISCUSSION

The first experiment reported above showed that phenytoin, 100 mg kg^{-1}, reduced food intake in pregnant rats for the first three days of administration but that subsequently weight gain proceeded normally. This dose of phenytoin, which seems high by clinical standards, actually produces in the rat a peak blood concentration of only 14·7 ± 2·3 $\mu g\ ml^{-1}$ (Elmazar 1978), which is within the clinical range in man. Close matching of food intake by pair feeding resulted in almost identical body weight gain patterns in the control and treated dams, so that it is unlikely that the effect observed on the foetuses were due to non-specific nutritional effects on the dams. The first experiment demonstrated that serum 5-HT in the controls increased progressively during the pre-weaning period reaching adult values by day 15. Similarly the brain 5-HT and 5-HIAA concentrations reached adult values by day 21. This result for the brain confirms earlier observations by Bourgoin et al (1974).

Since the serum 5-HT is largely due to accumulated platelet 5-HT, the increase of serum 5-HT concentrations (five-fold by age 15 days) may indicate that, the enzymatic processes responsible for the synthesis of 5-HT in the enterochromaffin and mast cells of the rats is still in the maturational phase during the pre-weaning period. Prenatal phenytoin treatment produced an increase in serum tryptophan and in brain tryptophan, 5-HT and 5-HIAA at 3 days of age, but thereafter these did not differ significantly from the controls. Since it has been reported that food deprivation in adult rats can produce a similar effect (Curzon et al 1972; Perez-Cruet et al 1972), it was obviously necessary to control for food intake in the neonatal rats. This was done by the fostering and cross-fostering procedures used in the second experiment. Here it could be seen that the treated pups reared by treated dams (Tt) when compared with the controls

(Cc) had the same or even more significant changes as seen in the first experiment. However, when the treated pups were reared by control dams (Ct) no difference was seen from control pups (Cc). This corresponded closely with the analysis of stomach contents with the Tt pups having the lowest quantity of milk in the stomach. It is interesting that the control pups reared by treated dams (Tc) also had a reduced milk intake and these were tending towards the same changes as seen in the Tt group. However as the changes in the Ct and Tc groups were both less than in the Tt group the effect was obviously not solely dependent on either the pups or the dams but on the interaction of both. Since the marked effects seen in the Tt group were prevented by one day's good nutrition (Ct pups) it is clear that the changes in serum and brain tryptophan metabolism induced by prenatal phenytoin were in fact due to impaired nutrition in the early post-natal period.

The increases in brain 5-HT and 5-HIAA observed are probably secondary to the increase in brain tryptophan which was observed. Brain tryptophan hydroxylase, the rate limiting enzyme for 5-HT synthesis, is normally unsaturated in adults with its precursor tryptophan (Eccleston et al 1965; Friedman et al 1972) and the increase in brain tryptophan concentration observed in starved adults has been proposed to cause the increase in brain 5-HT synthesis as indicated by the increase in 5-HIAA concentration with or without an increase in 5-HT level (Curzon & Knott 1974). This increase in brain tryptophan has been reported to be due to an increase in the free tryptophan in the blood (Knott & Curzon 1972). Bourgoin et al (1974), however, suggested that since all of the plasma tryptophan in neonatal rats is unbound, and brain tryptophan hydroxylase is near saturation under normal conditions, starvation should produce little further increase in brain 5-HT turnover. This has been shown in the present experiments to be incorrect since the serum and brain tryptophan contents were both increased and this resulted in an increase in 5-HT turnover as shown by the raised 5-HT and 5-HIAA content.

The raised tryptophan metabolism of treated rat pups at 3 days of age started to decrease at 4 days of age and was normal by 15 and 21 days of age. This can be explained by considering that these changes were dependent mainly on decreased lactation as discussed above. The maintenance of a suckling station is dependent on maturation of forelimb co-ordination of the young pups and on the behaviour of the dams which tend to retrieve straying young (nursing ability) (Rosenblatt & Lehrman 1963). According to Altman & Sudarshan (1975), spontaneous forelimb movement and co-ordination become frequent by 4 and 5 days of age. It seems that, at 3 days of age, maintaining a suckling station is mainly dependent on the mother but by 4 days of age as the forelimb co-ordination of the pups develops and matures, they start to be independently effective and they can struggle for access to milk from the mother. It is obviously important in rodent studies on neonatal brain amines to control for non-specific effects resulting from impaired maternal care during the first few days of life.

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