*Discussion.* The results of this study suggest that hyperglycemia may alter the pattern of steroid accumulation by isolated follicles in vitro. The most significant change was the increased endogenous production of progesterone by follicles from hyperglycemic rabbits. In view of the apparent increase in atretic follicles present in these rabbits (fig.) it could be postulated that the shift to progesterone production may be a result of this change in morphology. Similar findings have been reported in the hamster[10] and rabbit[11,12]. The induction of atresia by hyperglycemia is difficult to assess. It is possible that the deprivation of insulin may decrease gonadotropin support as seen in the rat[8] leading to degenerative changes in the ovarian follicle. Such a mechanism can also account for decreased ovulations and loss in weight of reproductive organs previously described[4-6]. Whether Alloxan has a direct toxic effect on the ovary could not be determined but it is expected that the time interval between drug administration and sacrifice would minimize acute drug effects.

1 This work was supported by the Medical Research Council of Canada. The technical assistance of Misses P. Dimond and J. Osoko is gratefully acknowledged. Dr B.R. Bhavnani provided the stimulus for this study.

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0014-4754/84/030289-03$1.50 + 0.20/0
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## Lack of long term effect of p-chlorophenylalanine on brain 5-hydroxytryptamine and electrocortical activity in conscious fetal sheep[1]

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*Summary.* Daily infusion of p-chlorophenylalanine (p-CPA) into unanesthetized fetal sheep for 3-6 days did not reduce brain 5-hydroxytryptamine (5-HT) concentrations or produce long-term changes in the pattern of electrocortical activity.

In fetal sheep the electrocorticogram (ECOG) differentiates into episodes of high and low voltage activity between approximately 115 and 125 days gestation; after this breathing movements only occur during the low voltage phase and occupy about 50% of the time[4]. 5-HT has been implicated in the genesis and control of high voltage (quiet) sleep in the adult[5], and infusion of the 5-HT precursor 5-hydroxytryptophan has been shown to prolong high voltage ECOG activity and increase breathing movements in fetal sheep[6]. To test the hypothesis that the appearance and maintenance of high voltage ECOG activity in the fetal lamb is due to the functional maturation of central 5-HT pathways we infused p-CPA, a substituted amino acid which depletes 5-HT stores by inhibition of the enzyme tryptophan hydroxylase, into unanesthetized fetal lamb in utero.

*Materials and methods.* Catheters were implanted into a carotid artery, jugular vein, trachea and amniotic sac of 6 fetal lambs (116-122 days gestation; term is 147 days) at a sterile operation under maternal halothane/$O_2$ anesthesia[7]. Pairs of stainless steel multistrand wire electrodes were implanted over the parietal dura to measure the ECOG, across 1 eye to record electro-ocular (EOG) activity, and into the nuchal, diaphragm and intercostal muscles to record the electromyograms (EMG's). ECOG and EOG activities, blood pressure, heart rate and breathing movements (from intratracheal pressure, diaphragm and intercostal EMG's) were recorded on a polygraph continuously for 9-15 days from the first day after surgery. Beginning on the 4th-6th post-operative days 600-700 mg p-CPA (as the methyl ester) dissolved 35-40 ml of warm 0.9% (w/v) NaCl was infused into the carotid artery at approximately 6 ml/h. One fetus received one infusion, but since this had no prolonged effect on ECOG or breathing the others were treated once daily for 3-6 days. 24 h after the final infusion (at 129-133 days gestation) the ewe was killed and the fetus removed from the uterus and weighed. Samples of brain tissue from 5 of the 6 fetuses were taken from the cortex (parietal) hippocampus, caudate nucleus, cerebellum, hypothalamus, pons, medulla and cervical spinal cord, placed in chilled tubes and immediately frozen. The samples were assayed for 5-HT and the metabolite 5-hydroxyindoleacetic acid (5-HIAA) within 7 days[8]. One fetus died in utero overnight and samples from that brain were not collected.

In addition to the brains of treated fetuses, samples were collected from a further 28 fetuses of gestational ages between 95 and 138 days and assayed for the indoleamines. This range of ages precedes and follows that during which the ECOG differentiates into high and low voltage activity[4].

Results are presented as mean ± SEM. The unpaired t-test was used to assess the effects of p-CPA treatment on brain concentrations of 5-HT and 5-HIAA.

*Results.* The concentration of 5-HT and 5-HIAA in the brains of control fetuses at 95 and 116 days, 122-127 days

Table 1. 5-Hydroxytryptamine (5-HT) and 5-hydroxyindoleactic acid (5-HIAA) concentrations (μg/g wet weight) in brains of fetal lambs 95–138 days gestation

| | Gestational age 95 days n = 6 | 116 days n = 7 | 122–127 days n = 10 | 130–138 days n = 10 |
|---|---|---|---|---|
| 5-HT | | | | |
| Cortex | 0.27 ± 0.04 | 0.22 ± 0.05 | 0.17 ± 0.02* | 0.16 ± 0.02* |
| Hippocampus | 0.37 ± 0.05 | 0.28 ± 0.07 | 0.28 ± 0.06 | 0.31 ± 0.05 |
| Caudate nucleus | 0.41 ± 0.08 | 0.20 ± 0.03* | 0.21 ± 0.05* | 0.17 ± 0.03** |
| Cerebellum | 0.44 ± 0.04 | 0.27 ± 0.05* | 0.21 ± 0.02* | 0.19 ± 0.03** |
| Hypothalamus | 0.64 ± 0.08 | 0.51 ± 0.13 | 0.34 ± 0.06** | 0.41 ± 0.07 |
| Pons | 0.44 ± 0.05 | 0.48 ± 0.12 | 0.41 ± 0.04 | 0.36 ± 0.06 |
| Medulla | 0.51 ± 0.07 | 0.48 ± 0.10 | 0.63 ± 0.20 | 0.31 ± 0.06* |
| Spinal cord | 0.89 ± 0.14 | 0.44 ± 0.12* | 0.53 ± 0.10 | 0.40 ± 0.07** |
| 5-HIAA | | | | |
| Cortex | 0.28 ± 0.05 | 0.22 ± 0.04 | 0.14 ± 0.03* | 0.25 ± 0.04 |
| Hippocampus | 0.45 ± 0.03 | 0.32 ± 0.05 | 0.30 ± 0.07 | 0.50 ± 0.08 |
| Caudate nucleus | 0.41 ± 0.07 | 0.28 ± 0.04 | 0.39 ± 0.18 | 0.33 ± 0.06 |
| Cerebellum | 0.30 ± 0.03 | 0.22 ± 0.03 | 0.18 ± 0.04 | 0.20 ± 0.03 |
| Hypothalamus | 0.51 ± 0.02 | 0.70 ± 0.17* | 0.53 ± 0.11 | 0.65 ± 0.08 |
| Pons | 0.47 ± 0.04 | 0.88 ± 0.16* | 0.70 ± 0.15 | 0.85 ± 0.14 |
| Medulla | 0.43 ± 0.03 | 0.57 ± 0.10 | 1.23 ± 0.60 | 0.72 ± 0.10* |
| Spinal cord | 0.48 ± 0.04 | 0.48 ± 0.14 | 0.42 ± 0.09 | 0.42 ± 0.07 |

Results shown as mean ± SE. * $p < 0.05$; ** $p < 0.01$ (t-test compared with values at 95 days gestation). Differences between 116 days and 122–127 days were not significant (t-test).

Table 2. Concentrations (μg/g wet weight) of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in various regions of the brain of 6 age-matched control fetal lambs and 5 p-CPA treated fetuses

| | 5-HT (μg/g) Control | Treated | 5-HIAA (μg/g) Control | Treated | Ratio 5-HIAA: 5-HT Control | Treated |
|---|---|---|---|---|---|---|
| Cortex | 0.18 ± .02 | 0.22 ± .04 | 0.19 ± .04 | 0.14 ± .02 | 1.18 ± .33 | 0.76 ± .26 |
| Hippocampus | 0.28 ± .07 | 0.60 ± .27 | 0.42 ± .10 | 0.21 ± .04 | 1.85 ± .64 | 0.53 ± .28 |
| Caudate nucleus | 0.31 ± .06 | 0.19 ± .04 | 0.58 ± .27 | 0.41 ± .33 | 1.51 ± .39 | 3.36 ± 2.75 |
| Cerebellum | 0.22 ± .01 | 0.30 ± .05 | 0.20 ± .05 | 0.12 ± .01 | 0.87 ± .47 | 0.48 ± .10 |
| Hypothalamus | 0.49 ± .09 | 0.45 ± .14 | 0.60 ± .17 | 0.31 ± .09 | 1.40 ± .42 | 0.85 ± .32 |
| Pons | 0.38 ± .05 | 0.34 ± .08 | 0.91 ± .27 | 0.38 ± .08 | 2.17 ± .57 | 1.48 ± .39 |
| Medulla | 0.85 ± .31 | 0.32 ± .10 | 1.88 ± .93 | 0.19 ± .02* | 1.67 ± .32 | 0.84 ± .32 |
| Spinal cord | 0.62 ± .11 | 0.64 ± .14 | 0.45 ± .16 | 0.29 ± .08 | 0.74 ± .20 | 0.54 ± .24 |

* $p < 0.05$ (t-test), treated compared to control. Values are means ± SEM.

and 130–138 days gestation are shown in table 1. At all gestational ages the highest concentration of both 5-HT and 5-HIAA were found in the brain stem and spinal cord. There was sometimes a large variation in 5-HT and 5-HIAA content between fetal lambs of the same age and even between twins and triplets. The concentration of 5-HT was highest in the youngest fetuses, and in the older groups levels in the cortex, caudate nucleus, cerebellum and spinal cord were significantly lower than those at 95 days gestation. However there were no changes in 5-HT levels between 116 and 127 days gestation, the period when the ECOG differentiates and breathing becomes episodic. Changes in 5-HIAA were small and did not show a significant trend with advancing gestational age.

Infusing p-CPA into fetuses at 125–132 days gestation caused only small reductions in 5-HT and 5-HIAA concentrations. The greatest reductions were in the medulla (table 2) although this was due partly to high levels of both 5-HT and 5-HIAA in a pair of twins in the control group. Larger changes were not associated with the greater number of infusions. However, treatment with p-CPA did cause a general reduction in the ratio of 5-HIAA to 5-HT concentrations in all brain areas except the caudate nucleus (table 2). Although these changes were not statistically significant the reduced ratio is consistent with the partial inhibition of 5-HT turnover (since this ratio expresses the

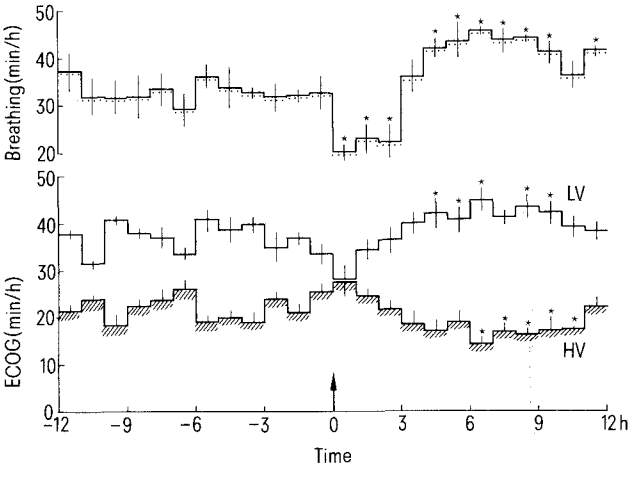


Figure 1. The number of min/h occupied by breathing movements, high voltage (HV) and low voltage (LV) electrocortical activity for the 12 h before and after infusion of 600–700 mg p-chlorophenylalanine to 5 fetal lambs. Infusion commenced at time 0. The data for 3–6 infusions on each animal have been combined. Value for each hour after start of infusion was compared to the mean of the 12 pre-infusion values (t-test; * $p < 0.05$). Vertical lines show the SE of the mean.

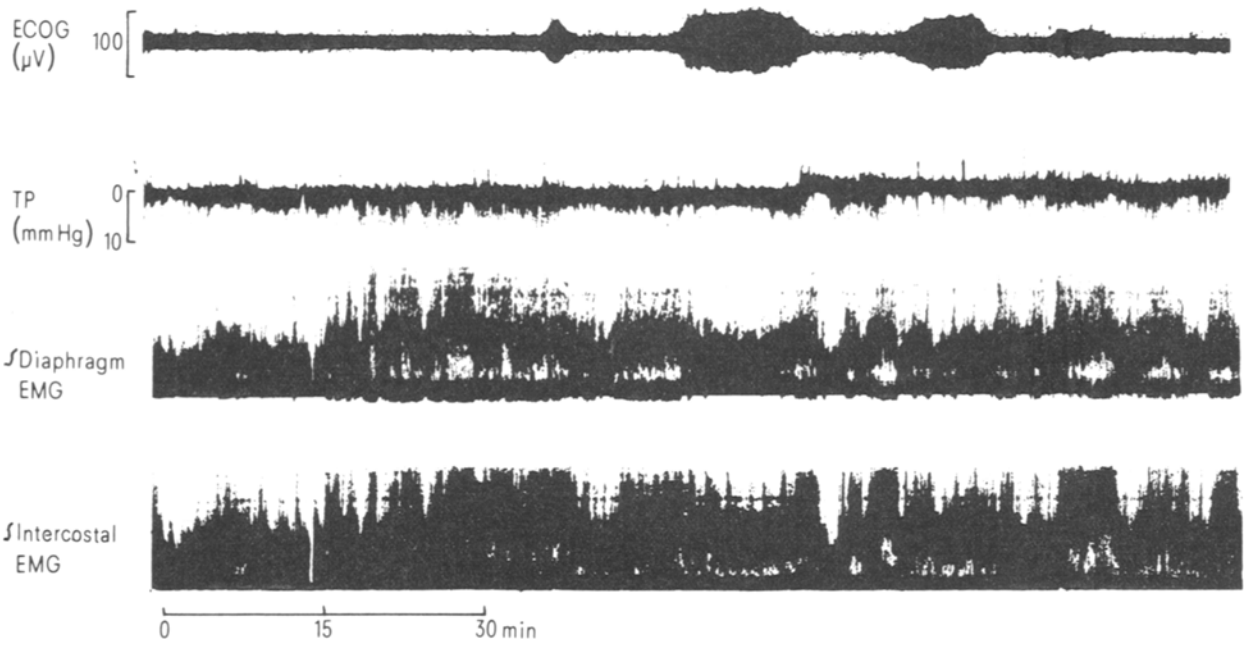


Figure 2. Record of electrocortical activity (ECOG), breathing movements (tracheal pressure, TP) and integrated diaphragm and intercostal electromyograms (EMG) from a fetal lamb (129 days gestation) 6 hours after commencement of a 30-min infusion of 600 mg of p-chlorophenylalanine.

dynamic relationship between the transmitter and its metabolite).

Infusing p-CPA had no long-term effect on the pattern of ECOG activity or breathing movements, although both were transiently altered during and shortly after each infusion. The amount of high voltage ECOG activity was reduced and low voltage activity increased in the 12 h following the start of each infusion (fig. 1). After each infusion breathing movements were at first decreased and then augmented in both depth and rate during the infusions. Periods of breathing lasting 4–6 h were observed; some of the breathing movements were present during high voltage ECOG (fig. 2). Fetal blood gases measured at the end of each infusion showed a consistent increase of $PCO_2$ (from $49.7 \pm 1.4$ to $66.7 \pm 3.8$ mm Hg; mean $\pm$SE) and fall of pH (from $7.36 \pm 0.01$ to $7.17 \pm 0.04$). 12–24 h after each infusion the pattern of ECOG activity and breathing movements, and the blood gases were normal.

*Discussion.* The levels of 5-HT and 5-HIAA were measured at different gestational ages because it was thought that changes in brain 5-HT might parallel the differentiation of the ECOG that occurs between 115 and 125 days gestation. However we found no change in 5-HT or 5-HIAA levels between 116 and 127 days gestation and 5-HT levels proved to be higher at 95 days than nearer term. This contrasts with the 4-fold increase in brain 5-HT shown in guinea-pigs during the last one-third of gestation when the ECOG is maturing[9]. The fall in brain 5-HT between 95 and 138 days, coupled with the relatively unchanged levels of 5-HIAA may, however, be indicative of increased turnover of 5-HT during this period.

The failure of these relatively large doses of p-CPA to reduce 5-HT levels significantly or to produce long-term alteration of ECOG activity in fetal lambs is surprising in view of the effectiveness of this amino acid in adult cats, dogs and rats[10–12]. While species differ in their susceptibility to p-CPA the dose that we used (3–6 infusions of 192 mg/kg based on fetal weight at post-mortem) is equal to or greater than the quantities usually administered. It has been suggested that the active compound which inhibits tryptophan hydroxylase is derived from hepatic metabolism of p-CPA after i.p. administration[10–13]. We administered p-CPA in the carotid artery so that the brain would receive a significant proportion of the total dose. After i.v. or i.p. injection much of the drug would enter the umbilical circulation and pass across the placenta to the mother. Our infusions were long (6–7 h) and a considerable quantity of p-CPA would have been presented to the hepatic circulation.

It is possible that the turnover of tryptophan hydroxylase in the brain of fetal lambs is greater than in the adult so that p-CPA caused only an incomplete and transient inhibition of the enzyme without significant alteration of 5-HT concentrations. The decrease in the ratio of 5-HIAA to 5-HT, although not statistically significant, is consistent with a partial inhibition of 5-HT synthesis and turnover. Also, the reduction in the amount of high voltage ECOG and increase in the amount of breathing suggests that serotonergic pathways are involved in both the genesis of high voltage activity and the inhibition of breathing movements at this time. But the alteration of ECOG activity and breathing movements was relatively transient and may not be entirely related to the ability of p-CPA to deplete 5-HT. Some effects of p-CPA on sleep patterns do not exactly parallel the decline in 5-HT concentrations[11,14,15] and the unexpected increase of arterial $PCO_2$ would be expected to alter breathing movements.

In summary, we have been unable to alter brain levels of 5-HT or to produce the sustained changes of the pattern of ECOG activity and breathing movements of fetal sheep with relatively large doses of p-CPA. Regional concentrations of 5-HT and 5-HIAA in the brains of fetal lambs do not change significantly during the portion of intra-uterine development when the distinctive pattern of breathing and high and low voltage ECOG activity emerges. The neurochemical mechanism(s) which determines this unique pattern may therefore not be closely related to activity of central serotonergic pathways.

1 Acknowledgments. This work was supported by an MRC grant to Professor G.S. Dawes, Nuffield Institute for Medical Research, Oxford. We thank C. Hanson and J. Deardon for help with the surgery and S. Lister for technical assistance.
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0014-4754/84/030291-04$1.50 + 0.20/0
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## Neocortical transplants in the rat brain: an ultrastructural study

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*Summary.* Electron microscopic analysis of neocortical transplants in the cerebellum of the host animals showed that the nerve cells, glial cells, and neuropil of the transplants were normal. These transplants showed anatomical integration with the host brain through various regions of interface. Neuropil interfaces were found to have a high density of synaptic profiles, and medullary interfaces had a very small number of synaptic profiles.

Various studies, in recent years, have shown that embryonic neural tissues can be transplanted into the brain of the laboratory mammals. The success of neural transplantation has been demonstrated by using thymidine-$H^3$ autoradiography[2], neuroanatomical staining methods[3,4], and histochemical techniques[5]. However, except for findings on connectivity of the transplants[6,7], there is no adequate information available on the ultrastructural characteristics of the transplants. This study is aimed at providing this information, and presenting characteristics of different types of interfaces between neural transplants and the host brain.

*Materials and methods.* Laboratory-bred Long-Evans hooded rats were used in this study. The donor embryos of 16-, 17- and 18-day stages were used to obtain neocortical tissue for transplantation. The host animals were 15-day-old on the day of surgery, and received transplants in midvermis of cerebellum via cisterna magna. Each host animal received only 1 transplant, and it was 3.0 $mm^3$ in volume. The techniques of obtaining embryos, dissecting them, preparing neural tissues, and transplanting them were identical to those described earlier[2,8].

The host animals were sacrificed 3.5 months after transplantation, during which time the transplants had grown and become fully differentiated. The animals were deeply anesthetized and perfused transcardially with a fixative composed of 4% glutaraldehyde and 2% paraformaldehyde in a 0.2 M cacodylate buffer (pH 7.4). After removing the brains, 1-mm-thick blocks from the transplants and from various regions of interface were prepared. They were washed in 0.1 M cacodylate buffer containing 0.1 M sucrose, postfixed in 2% osmium tetroxide with 0.1 M cacodylate buffer and 0.2 M sucrose, block-stained in 2% uranyl acetate for 2 h, and dehydrated and embedded in Epon-Araldite. At first thick sections were obtained, which aided in selecting specific structures for ultrastructural analysis. Regions from the middle of the transplants, cellular interface, medullary interface and neuropil interface were selected for further sectioning. The sections were studied under a Philips-300 electron microscope.

*Results. General comments.* In the brains of the host animals the neocortical transplants had not only grown large but also were anatomically integrated with the surrounding cerebellar parenchyma. There was no evidence of any pathological reaction in the transplants or the host brain, or of any glial scar formation between them. Three different types of interfaces between the transplants and the host brain could be identified, and they were: cellular interfaces, neuropil interfaces and medullary interfaces. The last 2 interfaces showed many striking differences, and therefore they were selected for a detailed analysis. Further, material prepared for light microscopy, available in the labaratory, that was processed for Nissl staining, Bodian-protargol staining and Golgi-Cox impregnation, was also studied. It provided a valuable perspective for the interpretation of ultrastructural observations. Since findings based upon light microscopic preparations have been presented elsewhere[4,9-11], they are not included in this report.

*Electron microscopic observations.* In the neocortical transplants large pyramidal cells (fig. 1A), stellate cells (fig. 1B) and various glial elements (fig. 1C) could be identified. The pyramidal and stellate cells had large pale nuclei located centrally in the somata. The cytoplasm surrounding these nuclei was relatively light, and contained various organelles such as Golgi apparatus, rough endoplasmic reticulum and mitochondria. In the case of the pyramidal cells the rough endoplasmic reticulum was more abundantly found. From the somata of these neurons dendrites were seen to emerge, and they were characterized by the presence of mitochondria, smooth endoplasmic reticulum and clusters of ribosomes. Generally, the transition zone between the cytoplasm and the trunk of a dendrite could be readily identi-