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BRIEF COMMUNICATION

Behavioral, Biochemical and Histological Effects of Prenatal Administration of Progesterone in the Rat

IAN R. COYLE

Department of Psychology, La Trobe University, Bundoora, Victoria, Australia 3083.

AND

ROBYN ANKER AND BRIAN CRAGG[1]

Department of Physiology, Monash University, Clayton, Victoria, Australia 3168

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COYLE, I. R., R. ANKER AND B. CRAGG. *Behavioral, biochemical and histological effects of prenatal administration of progesterone in the rat.* PHARMAC. BIOCHEM. BEHAV. 5(5) 587–590, 1976. – Pregnant Wistar rats were injected with progesterone (1.5 mg/kg) between Days 8 and 21 of gestation and the behavioral, biochemical and histological effects of this treatment were observed in the offspring. The progesterone offspring weighed less than the control animals during weaning and were retarded on one measure of exploratory activity in the open field. None of the other 29 tests used showed any significant difference apart from a 9% increase in the amount of brain DNA in the progesterone animals. It was concluded that these differences were fortuitous and that progesterone has no consistent or significant effects on brain development in rodents following prenatal administration.

Progesterone Prenatal administration Histological changes Biochemical changes Behavioral changes

RECENTLY there has been considerable interest in the effects of chemical agents administered during pregnancy on the behavior and brain development of the offspring. Most of this research has been concerned with subtle behavioral anomalies resulting from exposure to drugs during prenatal development [2–5, 9, 15, 16, 22].

The behavioral consequences of alterations in nutrition during prenatal and postnatal development have also been the subject of recent investigations. Much of this research has stressed the consequences of malnutrition during development [1,27]. However, a few papers have examined the effects of increased feeding during pregnancy. These showed that rats reared in small groups matured more quickly than control animals reared in litters of normal size [12, 18, 31]. In analogous experiments it has been reported that operative restriction of litter size in the rat and rabbit results in offspring that have a higher cerebral weight, higher total cerebral protein, and a higher amount of cerebral DNA than sham operated controls [29,30]. It has been suggested that this enhanced development may be due to a greater supply of nutrients to the fetus or a more efficient removal of waste materials [29]. Considering these findings it seems possible that any agent which facilitated nutrient supply to or waste transfer from the fetus could actually enhance prenatal development. Progesterone may be such an agent.

Although progesterone is a normal prerequisite of pregnancy, it has been suggested that increased levels of this steroid may enhance prenatal development by maintaining optimal functioning of the placenta. This notion is supported by the observation that babies tend to reach maturity more rapidly following administration of a progestin [19]. More recently, Dalton [7] has reported that the children of mothers administered progesterone during pregnancy (in order to prevent miscarriages) exhibited accelerated intellectual development as measured by academic success. Similar studies have been criticised on the basis that matching patients by social class, age, and order of parity does not control possible genetic factors. The present multidisciplinary study was undertaken to see whether effects analogous to those observed by Dalton [7] could be reproduced in the rat. The behavioral measures used were chosen on the basis of a previous study which has shown them to be sensitive indicators of behavioral development [3]. The histological measures used were brain weight, size and density of neurons and glial cells, content of DNA as a measure of total cellular density, and content of cholesterol as a measure of myelination.

METHOD

The animals were the male offspring of 18 naive Wistar

[1] Assisted by N. H. & M. R. C. of Australia.

rats which were 95–105 days old at the beginning of the experiment. A total of 111 offspring were used: 84 were evaluated on behavioral measures and 27 were evaluated on histological measures.

Prior to mating the females were housed 2–3 to a cage and kept in an air conditioned laboratory which was maintained at approximately 22°C. The daily light cycle was 12 hr light, starting at 7.00 a.m., and 12 hr dark. The females were matched on the basis of weight, assigned to either a progesterone or placebo group and placed in a mating cage together with a male rat of proven fertility. The female was removed to a breeding cage when a vaginal plug was observed and this was taken as day 0 of gestation. Drug administration was started on day 8 of gestation and continued daily until day 21 of gestation. The progesterone group was administered a 1.5 mg/kg dose of SC progesterone (0.4 g/100 ml dissolved in olive oil) and the Placebo group was administered a similar dose of olive oil. All drugs were administered subcutaneously at about the same time each day. This dosage was comparable to that administered to Dalton's [7] high dosage group, whose mothers received at least 100 mg/daily of progesterone during pregnancy.

The values reported in the literature of peripheral plasma concentrations of progesterone in pregnant rats and humans vary considerably. However it seems likely that the endogenous plasma levels of progesterone during the second and third trimesters in humans and the analogous period in the rat are approximately equivalent [17, 19, 20, 23, 28]. In the present study the dose of exogenous progesterone would have produced an increase of 35–40% of the plasma progesterone levels observed on Days 8–9 of pregnancy in the rat [23,28]. By Days 15–16 of pregnancy this dose would have amounted to a 20% increase, rising to an 83–125% increase at Days 20–21 [23,28]. (These figures are conservative estimates and are based on a blood volume of 6% of body weight [8]).

Throughout the drug injection period and the subsequent evaluation the experimenters were unaware of the drug treatment given specific animals (the drug and control substances were identified by a code during this period).

On the day of birth, the litters were reduced to 8 offspring retaining the maximum number of males per litter. If there were less than 5 offspring alive in a litter by 22 days after birth the data from that litter were discarded. On Day 0 (the day of birth) all offspring were marked for identification by clipping a single joint from one of the forefeet. Toe marking was carried out at the completion of testing on Day 0.

The apparatus and procedures used for behavioral testing have been described in detail elsewhere [3]. Briefly, the animals were inspected for the maturation of physical features and reflex ontogenesis; exploratory behavior was evaluated in an open field; and spontaneous alternation was tested in a T-maze. Testing of physical maturation and reflex ontogenesis took place when the animals were between 1 and 18 days, open field behaviour was observed at 9, 13, 17 and 21 days and spontaneous alternation was tested when the animals were 21 days old.

Rats that had not been used for behavioral testing were killed and fixed for perfusion at 37–38 days. These rats were littermates of those tested behaviorally: 13 were progesterone offspring and 14 were placebo offspring. At autopsy, the rats were weighted, anesthetized with ether, and perfused through the left cardiac ventricle with 4% formaldehyde and 1% glutaraldehyde (in 0.1M sodium phosphate buffer at pH 7.4) at a steady pressure of 100 mm Hg. The brains were removed with olfactory bulbs intact, cut from the spinal cord at C1, and weighed. The olfactory bulbs, cerebellum and brainstem immediately behind the inferior colliculus were cut off, and the brain reweighed. The maximal width in the coronal plane and maximal length in the parasagittal plane were recorded. The brains were then cut in half on the midline, and the left side weighed and used for biochemical estimations, while the right side was sectioned to count neurons.

Weighed brain samples were stored at −4°C until required for analysis. Each half forebrain was homogenized in cold 10% trichloracetic acid to give a 10% homogenate, the final volume being recorded. Cholesterol was measured in three separate aliquots of 0.1 ml by the method of Rudel and Morris [20], and the three results averaged. Cholesterol (Hopkins and Williams) was used to standardise the measurements. From the remaining homogenates the acid-soluble compounds and lipids were successively extracted, and the nucleic acids dissolved out in hot TCA. DNA was estimated by the colourimetric reaction described by Schneider [26]. The standard used was DNA sodium salt (highly polymerised) derived from salmon testes (BDH).

Frozen sections of the frontal cortex and the dorsal hippocampal region were cut in the coronal plane at 20 μ and mounted from the fixative onto the gelatinised slides. After staining with cresyl violet, neuronal and glial nuclei were counted in scans of the cortex from layer II to the white matter. The diameters of the neuronal nuclei were measured in order to estimate the density of neurons. Details of the methods have been described previously [6].

RESULTS

Prenatal exposure to progesterone had no significant effects on reproductive success, neonatal mortality, physical maturation, reflex ontogenesis and spontaneous alternation. Indeed, the median values of the drug and control groups on these measures were identical in all but one case (the median number of live offspring per litter in the progesterone and placebo groups were 11 and 12 respectively: this difference was not significant).

The open field data were analysed by a test for trend [9]. The only significant difference between the progesterone and placebo offspring was in the occurrence of head lifting responses when between group means were considered. In general the drug-exposed animals displayed less head lifting than the control offspring ($p<0.01$). However, these differences were more apparent in the early stages of testing than later, as is indicated by the significant trend differences between groups and cubic trend differences ($p<0.01$ and 0.05 respectively). The differences between the drug and control offspring with respect to head lifting were parralleled when body weight during weaning was evaluated. The progesterone offspring weighed less than the placebo offspring in general ($p<0.05$) but these differences become less apparent with increasing age as indicated by significant between groups and linear trends ($p<0.01$ and 0.05 respectively). This latter observation was supported by an independent analysis of the body weights of the animals used for histological evaluation at 37–38 days of age which failed to show any significant differences between the drug-exposed and control offspring.

To give an idea of the extent of the similarity between

TABLE 1

HISTOLOGICAL AND BIOCHEMICAL ANALYSIS OF PROGESTERONE AND PLACEBO OFFSPRING

Dependent Variable	Progesterone	Placebo	p
Mean brain weight*	1.68 ± 0.03 g	1.66 ± 0.02 g	NS
Mean brain weight†	1.25 ± 0.02 g	1.23 ± 0.01 g	NS
Number of neurons per scan	171.0 ± 6.1	176.8 ± 5.1	NS
Number of neuroglia per scan	108.6 ± 4.4	116.1 ± 5.9	NS
Ratio of neurons to neuroglia	1.59 ± 0.1	1.61 ± 0.9	NS
Neuronal diameter	16.3 ± 0.15μ	16.7 ± 0.33μ	NS
Neuronal densities ($x10^7/cm^3$)	4.28 ± 0.2	4.34 ± 0.1	NS
Brain cholesterol (μg/mg brain)	22.3 ± 0.4	23.2 ± 0.5	NS
Brain DNA (μg/mg brain)	1.233 ± 0.046	1.125 ± 0.020	$p<0.02$

All the data were analyzed by Students t-test. Mean and SEM stated.
NS: Not significant with $\alpha = 0.05$.
*Including olfactory bulbs, brain stem and cerebellum.
†After removal of olfactory bulbs and cerebellum and cutting through the medulla immediately behind the inferior colliculus.

the drug and control offspring the histological and biochemical analysis of the two groups is summarized in Table 1. The only significant difference between the two groups was a 9% increase in the amount of DNA in the drug-exposed offspring.

DISCUSSION

Of the 29 parameters measured only three showed any significant difference between the drug-exposed and control offspring, and one in 20 of the tests would be expected to be significant at the 5% level by chance. Thus, the present investigation suggests that prenatal administration of progesterone does not have any consistent effects upon CNS development in the rat, a finding that is in agreement with a previous observation [32]. However, before this conclusion can be reached there are several factors that must be considered.

It has been reported [13,14] that administration of progesterone to pregnant rats late in gestation inhibits lactation. Since the offspring were not fostered in the present study it might be argued that the failure to observe any beneficial effects resulting from progesterone treatment may be due to a suppression of lactation. Certainly the decreased body weight exhibited by the progesterone treated offspring is consistent with this explanation. However, suppression of lactation has only been observed [13,14] with higher doses of progesterone than were used in the present study (approximately 3 mg/kg as compared to 1.5 mg/kg). Furthermore they have reported that inhibition of lactation only occurs if the drug is administered on day 23 of gestation (i.e. near parturition): in the present study injections of progesterone ceased on day 21 of gestation, at least one day prior to parturition.

The possible effects of prenatal administration of progesterone on the developing brain have not been extensively studied. This particularly applies to rodents and the failure of the present study to confirm Dalton's [7] observations may arise from the fact that the rat is not a close embryological model to man. Alternatively, pharmacological considerations may underlie the failure to find any significant differences between the drug-exposed and control animals. Previous studies concerned with the metabolism and physiological effects of progesterone in the pregnant rat have utilised higher doses than those used in the present study [11, 13, 14, 21]. However, a recent study has indicated that 1.3 mg/kg of progesterone, administered during the second and third trimesters of pregnancy, reduces defecation in the open field in rats (Stevens, upublished observations). It is, then, probable that the dosage used in the present study was sufficient to induce beneficial effects on behavioural and physiological development, if progesterone has any such effects. Furthermore, it is apparent that the developing central nervous system is particularly sensitive to the effects of pharmacological agents administered during pregnancy [2, 3, 4, 5, 8, 15, 16, 22]. Thus, while it is possible that beneficial effects on brain development might be found in rodents with higher dose levels of progesterone it is considered unlikely in view of the current evidence.

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