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Interruption of pregnancy by barbiturates in albino rats

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Abstract. Barbiturates inhibit the LH surge and release of gonadotrophins (LH and FSH) and prolactin from the pituitary in rats and hamsters. In the present study administration of phenobarbital (7·5 mg) or barbital sodium (20 mg) twice a day from day 8–11 interrupts the pregnancy in rats with little or no foetal survival. The ovaries and the uterus of these rats resemble those of non-pregnant rats when autopsied on day 20 of pregnancy. These results suggest that the failure of maintenance of pregnancy by barbiturate treatment may be due to the inhibition of luteotrophic hormones from the pituitary during the crucial period of pregnancy, resulting in the insufficient secretion and release of ovarian progesterone and also estrogen.

Keywords. Gonadotrophins ; pituitary ; phenobarbital ; barbital sodium ; luteotrophins ; corpora lutea ; foetuses.

1. Introduction

Maintenance of pregnancy is the consorted efforts of all endocrine glands mediated through the hypothalamo-hypophyseal-ovarian and placental axes. Hypophysis is indispensable during first half of pregnancy, but the ovaries are essential throughout the gestation, as the placental gonadotrophins take over the functions of the pituitary gonadotrophins during later part of pregnancy in rats (Lyons and Ahmad 1973 ; Rothchild *et al* 1974). Maintenance of pregnancy by exogenous LH or other luteotrophins in hypophysectomized rats, and by proper doses of progesterone and estrogens in ovariectomized rats and hamsters is achieved by several investigators (Jagannadha Rao *et al* 1972 ; Yoshinaga *et al* 1972).

Studies with pheno- and pentobarbital indicate that these drugs inhibit the pituitary LH surge and tonic release of FSH, LH and prolactin, and also interfere with the ovarian steroidogenesis directly (Gupta and Karavolas 1973 ; Norman *et al* 1973 ; Blake 1974). As barbiturates interfere with much of the endocrine activities of pituitary and ovary that are essential for the maintenance of pregnancy, the aim of the present investigation was to study the effects of phenobarbital and barbital sodium on pregnancy in rats.

2. Material and methods

Normal cycling, nulliparous rats of Holtzman strain, weighing 140–150 g, 80–90 days old were mated with fertile males at proestrus or early estrus. The rats showing spermatozoa in the vaginal smears on the subsequent day were selected for experimentation and that day was designated as day 1 of pregnancy. The selected rats were laparotomized under mild ether anaesthesia on day 8 of pregnancy to note the number of implantations and those having normal implantations were taken for the further experimentation.

2.1. *Experiment I*

To study the different dose effect of phenobarbital ; 2·5 mg, 5·0 mg or 7·5 m, phenobarbital/100 g body weight, in 0·5 ml saline was injected subcutaneously twice a day from day 8 through day 19 of pregnancy.

2.2. *Experiment II*

To study the different dose effect of barbital sodium 10 mg, 15 mg or 20 mg barbital sodium/100 g body weight in 0·5 ml saline was injected subcutaneously, twice a day from day 8 through day 19 of pregnancy.

For the above experiments, saline treated controls were maintained. The drug treatment was continued until profuse vaginal bleeding was observed. All the rats were autopsied on day 20. The number of foetuses, placentomas, placental scars and placentae were recorded. Ovaries were weighed, fixed in Bouin's fluid, embedded in paraffin, sectioned and stained in haematoxylin-eosin for histological observations. The rats were maintained in individual cages with Hindustan Lever rat feed and water *ad libitum* at a room temperature of 27± 1 °C with 12 hrs of lighting schedule.

3. Results

3.1. *Interruption of pregnancy (tables* 1 *and* 2)

Administration of different doses of phenobarbital or barbital sodium,twice a day from day 8 through day 19 interrupts the pregnancy in rats to various levels. Low doses of phenobarbital i.e. 2·5 mg/100 g body weight interrupts pregnancy in 1/5 rats, while 5 mg of the same drug causes partial maintenance in 2/5 rats and the remaining rats in these groups exhibit successful maintenance of pregnancy to full term.

Similarly 10 or 15 mg barbital sodium is not effective in interrupting the pregnancy wherein 5/6 rats or 5/5 rats maintain the pregnancy completely up to day 20. Only one rat with 10 mg barbital sodium exhibits partial maintenance.

These results indicate that the phenobarbital is more potent than barbital sodium in affecting the pregnancy even in low doses. The effective dose of phenobarbital or barbital sodium in interrupting the pregnancy is 7·5 mg or 20 mg respectively wherein 9/9 rats or 8/9 rats show complete abortion with profuse vaginal bleeding on day 12 or 13 of pregnancy. In saline treated controls, the pregnancy is maintained successfully in almost all rats.

Table 1. Effect of graded doses of phenobarbital on pregnancy in rats.

Treatment—day 8–19 Dose/100 gms body wt. 2 doses/day

Treatment	Mean in relation to pregnant at laparotomy M ± S.E.				% foetal survival	Ovarian wt. mg/ 100 gms body wt. M ± S.E.
	Implantations	Placental scars	Placentomas	Live foetuses		
Control (5)	7·60 ± 1·51	0·2 ± 0·2	...	7·40 ± 1·51	97·4	39·89 ± 4·19
Phenobarbital 2·5 mg (5)	7·40 ± 0·67	1·40 ± 1·39	...	6·20 ± 1·79	83·8	40·61 ± 7·75
5·0 mg (5)	8·60 ± 0·51	0·8 ± 0·37	...	7·60 ± 0·56	88·4	40·23 ± 1·37
7·5 mg (9)	7·50 ± 0·59	...	...	...	0·00	31·17* ± 1·68

Laparotomy is done on day 8 and autopsy on day 20 of pregnancy.
Number in paranthesis denotes the number of rats.
M ± S.E.= Mean ± Std. error. * $P < 0·05$.

3.2. *Foetal survival*

The percent foetal survival is calculated in relation to number of live foetuses on day 20, with reference to the number of implantations observed on day 8, at laparotomy. In saline treated controls 37 foetuses were found out of 38 implantations indicating 97·4% foetal survival. With 2·5 mg or 5·0 mg phenobarbital treatment 83·8% or 88·4% respective foetal survival is observed. But with 10 mg or 15 mg barbital sodium administration the respective foetal survival is 97·9 or 100·0% which is almost similar compared to that of controls. However, 7·5 mg phenobarbital or 20 mg barbital sodium administration, the implantation loss is considerable, wherein the number of foetuses *vs* implantation sites is 0/60 or 7/63 respectively, thereby indicating that foetal survival is nil or 11·1% with respective to drug treatment.

The above results indicate that phenobarbital is more potent in its litter destroying effect than barbital sodium, which may be due to its prolonged action on the central nervous system.

Table 2. Effect of graded doses of barbital sodium on pregnancy in rats.

Treatment-day 8–19 Dose/100 gms body wt. 2 doses/day

Treatment	Mean in relation to pregnant at laparotomy M ± S.E.				% foetal survival	Ovarian wt. mg/ 100 gms body wt. M ± S.E.
	Implantations	Placental scars	Placentomas	Live foetuses		
Control	7·60	0·2	...	7·40	97·4	39·89
(5)	±	±		±		±
	1·51	0·2		1·51		4·19
Barbital sodium	7·83	0·17	...	7·66	97·9	36·60
10 mg	±	±		±		±
(6)	0·48	0·17		0·37		1·50
15 mg	8·20	...	...	8·20	100·0	42·2
(5)	±			±		±
	0·36			0·36		3·66
20 mg	7·00	...	...	0·78	11·1	25·59**
(10)	±			±		±
	0·41			0·78		1·46

Laparotomy is done on day 8 and autopsy on day 20 of pregnancy.
Number in paranthesis denotes the number of rats.
M ± S.E. = Mean ± Std. error. ** $P < 0·01$.

3.3. *Gravimetric and histological changes of the ovary*

In the controls, where the pregnancy is maintained to full term, the ovaries are large with well developed corpora lutea, weighing 38·89 mg. With 2·5 mg or 5·0 mg phenobarbital treatment, wherein pregnancy is not much affected, the ovary exhibits large well developed corpora lutea similar to those of controls. However in the rats treated with 7·5 mg phenobarbital or 20 mg barbital sodium, where the complete abortion has occurred, with almost nil foetal survival, the ovaries are small with moderate sized corpora lutea and ovulated follicles. The ovaries are reduced significantly weighing 37·17 mg ($P < 0·05$) or 25·59 mg ($P < 0·01$) with the administration of phenobarbital or barbital sodium respectively. These aborted rats come to estrus within 3–4 days after profuse vaginal bleeding and hence the ovaries resemble those of nonpregnant rats. These results indicate a good correlation between the percent foetal survival, ovarian weight and its histology.

The adverse effect of barbiturates on pregnancy seems to be due to blockade of pituitary gonadotrophins release during the critical period of gestation (day 8–11),

wherein the pituitary hormone balance is essential for the normal functioning of the ovaries which are responsible for the maintenance of gestation during its first half.

4. Discussion

The apparent neutralization of LH during days 7–11 of pregnancy in rats results in the termination of gestation by foetal resorption (Rothchild *et al* 1974). Initiation of a rise in the progesterone synthesis and pituitary LH release coincides with an increased follicular growth and hypertrophy of corpora lutea between day 9–12 of pregnancy in rats and hamsters (Greenwald 1973 ; Rothchild *et al* 1974). Therefore it is evident that pituitary LH is essential for the maintenance of corpora lutea in the functional state as to produce progesterone, sufficient to maintain the pregnancy during the early half. In the present investigation, phenobarbital (7·5 mg) or barbital sodium (20 mg) causes profuse vaginal bleeding with foetal loss in 8/8 or 8/9 rats respectively when treated from day 8–11. The ovaries of these rats are significantly reduced with very small corpora lutea and resemble to those of non-pregnant rats when observed after autopsy on day 20. The probable *modus operandi* is the continued inhibition of pituitary LH release during the crucial period of pregnancy by the chronic treatment of barbiturates, as these drugs are known to inhibit the LH surge and release in rats and hamsters (Norman *et al* 1973 ; Blake 1974 ; McCormack 1974). Therefore for all probabilities, the ovaries of barbiturate treated rats may not be functional due to LH inhibition, as LH stimulates the production of progesterone from corpus luteum, and Yoshinaga *et al* (1972) and Jagannadha Rao *et al* (1972) have observed a decrease in the progesterone and 20α—OH—P by neutralizing endogenous LH by LH antiserum treatment. Therefore the corpora lutea of pregnant rats seem to be dependent upon LH to maintain the high progesterone levels during gestation. It can be postulated that interruption of pregnancy by chronic treatment of barbiturates is due to continued blockade or lowering of LH, resulting in subnormal production of progesterone. Besides, it has been reported that barbiturates interfere directly with the ovarian steroidogenesis by decreasing the 3β–hydroxy steroid dehydrogenase activities (Gupta and Karavolas 1973).

It is also stated by Greenwald and co-workers (1973, 1974) that prolactin with FSH or estrone forms the luteotrophic complex during the early part of pregnancy. These luteotrophins might have been decreased in barbiturate treated rats, as barbiturates inhibit both gonadotrophins (FSH and LH) and prolactin release (Ajika *et al* 1972 ; Beatti *et al* 1973). The ineffectiveness of low doses of these drugs causing abortion or foetal resorption may be due to their failure in inhibiting the pituitary gonadotrophins and prolactin effectively. Therefore it can be concluded that the interruption of pregnancy in barbiturate treated rats is not only because of the decreased pituitary gonadotrophins and prolactin release during the crucial period, but also due to the direct interference of these drugs in the ovarian steroidogenesis.

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References

Ajika K, Kalra S P, Fawcett C P, Krulich I and McCann S M 1972 The effect of stress and nembutal on plasma levels of gonadotrophins and prolactin in ovariectomized rats; *Endocrinology* **90** 707–715

Beattie C W, Campbell C S, Nequin L Q, Soyker L F and Schwartz N B 1973 Barbiturate blockade of tonic LH secretion in the male and female rats ; *Endocrinology* **92** 1634–1638

Blake C A 1974 Localization of the inhibitory action of the ovulation blocking drugs on release of luteinizing hormone in ovariectomized rat ; *Endocrinology* **95** 999–1004

Greenwald G S 1974 Modification in ovarian and pituitary function in the hypophysectomized pregnant hamster ; *J. Endocrinol.* **61** 35–51

Greenwald G S 1973 Further evidence for a luteotrophic complex in hamster ; Progesterone determination of plasma and corpora lutea ; *Endocrinology* **92** 235–242

Gupta C and Karavolas R J 1973 Lowered ovarian conversion of 14 C-pregnenolone and other metabolites during phenobarbital (PB) block of PMS-induced ovulation in immature rats ; inhibition of 3β-hydroxy steroid dehydrogenase ; *Endocrinology* **92** 117–124

Jagannadha Rao A, Madhwa Raj H G and Moudugal N R 1972 Effect of LH, FSH and their antisera on gestation in hamster ; (*Mescricetus auratus*) ; *J. Reprod. Fert.* **29** 239–249

Lyons W R and Ahmad N 1973 Hormonal maintenance of pregnancy in hypophysectomized rats ; *Proc. Sec. Exp. Biol. Med.* **142** 198–202

McCormack C E 1974 Reversal by progesterone of barbiturate blockade of ovulations : effect of concentration of serum LH (38098) ; *Proc. Soc. Exp. Biol. Med.* **146** 329–332

Norman R L, Blake C A and Sawyer C H 1973 Evidence for neural sites, of action of phenobarbital and progesterone on LH release in the hamster ; *Biol. Reprod.* **8** 83–86

Rothchild I, Pepe G J and Morishige W K 1974 Factors affecting the dependency on LH in the regulation of corpus luteum progesterone secretion in the rat ; *Endocrinology* **95** 280–288

Yoshinaga K, McCdonald G J and Greep R O 1972 Influence of various doses of LH on foetal survival in hypophysectomized rats ; *Proc. Soc. Exp. Biol. Med.* **140** 193–195