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STUDIES ON THE MECHANISM OF NIDATION

XXXI. FAILURE OF ERGOCORNINE TO INTERRUPT GESTATION IN THE RAT IN THE PRESENCE OF FOETAL PLACENTA

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Summary. A single administration of ergocornine methanesulphonate (ECO) interrupts pregnancy of the rat when given on any day until the 7th after coitus, but not later. The drug also interrupts pseudopregnancy at any time during its course, without temporal limitation. The failure of ECO to interfere with pregnancy is correlated with the presence and endocrine activity of an implanted conceptus.

The factors causing failure of ECO to interfere with gestation after implantation were identified by a series of successive steps: (1) The presence of deciduomata—thought to be analogous to maternal decidual tissue—in the pseudopregnant uterus was shown not to oppose ECO action. (2) Animals, whose foetuses have been removed, leaving only maternal and foetal placenta *in situ*, were protected against the action of ECO. Thus, this implicated by elimination the foetal placenta as causing failure of ECO action in pregnancy after the 7th day. Indeed, placental grafts from late pregnant donors could be shown to overcome ECO action even in this first 7-day period.

As early as the 10th day, a luteotrophic influence was present in placental extracts, as measured by prevention of ECO action on pregnancy. In the literature, a luteotrophic influence is attributed to (foetal) placental tissue. The failure of ECO to interrupt pregnancy by the 8th day is taken as indication of placental luteotrophic activity at that time, i.e. 3 days before it can be detected when hypophysectomized rats are used for this purpose.

The action of ECO is considered as mediated by pituitary LTH depression. Thus, the use of this drug allows suppression of LTH without performing hypophysectomy. Placental luteotrophin is not affected by ECO.

INTRODUCTION

Certain ergot alkaloids interrupt early pregnancy of the rat and cause onset of oestrus within 2 to 3 days after their subcutaneous administration (Shelesnyak,

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1955). One of the alkaloids studied, ergotoxine, was a mixture of three alkaloids (Stoll & Hofmann, 1943); of these, ergocornine (ECO) has been utilized most in recent research. Shelesnyak (1955) noted "that the period of pregnancy when ergotoxine is effective . . . is limited to the first seven days post-coitus . . . ; those (rats) who received ergotoxine after day eight post-coitus delivered normal litters".

ECO was thought to interfere with the availability of progesterone. Administration of exogenous progesterone, made available during and after ergotoxine, could overcome the interruption of pregnancy by ergotoxine injection (Shelesnyak, 1956); a course of exogenous prolactin was also effective (Shelesnyak, 1958). Since prolactin induces the ovary to secrete progesterone, the ergotoxine had apparently not interfered with the ovarian capacity to respond to luteotrophic activity.

There are other indications suggesting that ECO may be interfering with the luteotrophic regulation of ovarian function. Rats with auto-transplanted, anterior pituitary glands responded to ECO—under different experimental conditions—in the same way as females with intact pituitary glands, indicating that direct ECO action on the hypophysis causes interference with luteotrophic activity of that gland (Zeilmaker & Carlsen, 1962; Varavudhi, 1965; Kisch, unpublished).

In the rat, maintenance of gestation depends on the presence of functional corpora lutea which can be maintained only by continuously available luteotrophic stimulation. Initially, the hypophysis ensures this luteotrophin; however, during the course of gestation, the placenta becomes a major source of luteotrophic hormone (for review, see Amoroso, 1960). The exact time when the placenta acquires this particular endocrine function in the rat is not known.

This study was designed to assess whether the failure of ergot alkaloids to interfere with pregnancy after the 7th day was due to the presence of, and luteotrophic secretion by, the foetal placenta at that time. This assumption may be valid if ECO does not act on the foetal placental luteotrophin secretory mechanism.

MATERIALS AND METHODS

Virgin female rats of the Biodynamics Institute colony, approximately 100 days old, were used. One hundred and twenty-three females (114 for experimental evaluation; nine donors for biological material) were mated with males of proven fertility; and impregnation was assumed on finding spermatozoa in the vaginal smear on the morning after mating (Day L_0 of pregnancy). In this colony more than 97% of inseminated females became pregnant. In thirty-two animals (thirty for testing; two as donors of biological material), pseudopregnancy was induced by electrical stimulation of the cervix uteri during oestrus. The 1st day of leucocytic smear is designated L_1 of pseudopregnancy. In twenty-two of these, deciduoma was induced on the morning of L_4 by scratching both uterine horns longitudinally with a needle.

Vaginal smears were examined daily, before and during the course of the experiment. Ergocornine methanesulphonate (Sandoz) was administered sub-

cutaneously in 0·25 ml of 70% aqueous ethanol. The dose was 1 mg/rat, except where noted otherwise. All surgical procedures were performed while the experimental animal was under ether anaesthesia.

EXPERIMENTAL AND RESULTS

Time limits of effectiveness of ECO in pregnant animals

The time limits for ECO interference with gestation were determined by administering a single injection of 1 mg of ECO on one of the days L_4, L_5, etc., to L_{10} inclusive, to ten animals for each day of pregnancy.

The groups treated on L_4, L_5 or L_6 were killed 4 days after injection and the uteri examined for evidence of implantation. Groups treated on L_7 to L_{10} were autopsied no earlier than 4 days after ECO, and varied up to 10 days after injection. This delay allowed observation of foetal heart beat as confirmation of successful maintenance of pregnancy and viability of the implants. Five rats of the group which received ECO on L_9 were allowed to go to term to study possible late effects on the mechanism of delivery and subsequent suckling.

ECO is effective when administered up to L_6 of pregnancy (Table 1), as evidenced by the presence of oestrous vaginal smears within 2 to 3 days, and

TABLE 1

THE EFFECT OF A SINGLE INJECTION OF ERGOCORNINE METHANESULPHONATE (ECO) ADMINISTERED TO PREGNANT RATS

*Treatment** (*Day of pregnancy*)	*No. of rats with interrupted pregnancy/ten females*
L_4†	10
L_5	10
L_6	9
L_7	1
L_8	2
L_9	0
L_{10}	0

* 1 mg ECO in 0·25 ml 70% ethanol subcutaneously.
† Day L_0 is the day after mating.

empty uteri at autopsy. When administered on L_7 or later, the ECO is virtually ineffective in interfering with gestation; pregnancy was maintained and viable foetuses were observed in all but three cases. The five animals which received ECO on L_9 were followed till term, when they were delivered normally and suckling proceeded unimpaired during the first 3 days, after which no observations were made.

Thirty-eight rats remained pregnant; all but five had a continuous leucocytic vaginal smear record. These five exhibited oestrous vaginal smears following the ECO injection. These animals were randomly distributed among the groups.

In an attempt to test dose-dependency on the time of response, a group of animals was injected on L_7, the day of onset of the period of ECO ineffectiveness, with three times the ED_{99}. The 3-mg doses failed to interrupt pregnancy in all eight females.

Determination of factor(s) causing failure of ECO to interfere with pregnancy after implantation

A significant difference between the pregnant and pseudopregnant rat is the conceptus, i.e. the maternal and foetal placental components and the embryo.

To test the possibility that the presence of maternal placental tissue, i.e. the decidua, was the basis for ECO failure after implantation, the role of 'pure' maternal decidua was examined. This was done by testing ECO in pseudopregnant rats bearing induced deciduomata.

Administration of ECO to ten regular L_8 pseudopregnant animals caused the appearance of vaginal oestrus within 2 to 3 days. Administration of ECO to pseudopregnant rats bearing massive deciduomata, on L_8 or L_{10} revealed the appearance of oestrus within 2 to 3 days in ten out of ten females in each group. Thus, there was no evidence that presence of decidual tissue prevented ECO action. In all cases, ECO interrupted the pseudopregnant state before its natural termination. In this laboratory, normal pseudopregnancy is characterized by persistence of leucocytic vaginal smear for 14 days (range 11 to 18); and in the presence of extensive deciduomata this period is prolonged beyond 15 days.

On the basis that the deciduoma resembles the maternal placenta closely, functionally as well as structurally, the fact that it did not inhibit ECO action pointed to the foetal placenta and/or the embryo itself as responsible for ECO inhibition.

To test the possibility that the foetal placental tissue was the responsible agent for ECO failure, the drug was given to females whose uteri contained only placental tissue, but no foetuses.

Animals were prepared as follows: on L_{12} of pregnancy embryos were removed through a small incision of the uterus at each implantation site between mesometrium and antimesometrium. The foetus was gently expressed through the incision; foetal and maternal placenta remained. The uterus was not sutured.

The untreated controls (foetuses removed, no ECO) and all six of the ECO-treated rats showed persistent leucocytic vaginal smears for the duration of a normal pregnancy (21 to 22 days) and then oestrus followed.

Thus, ECO failed to interfere with pregnancy after implantation, even in the absence of the foetus. As deciduoma is equated with the maternal component of the placenta, the only difference between this experimental group and that consisting of pseudopregnant animals bearing deciduomata in their uteri is the presence of a foetal component of the placenta. It appears that the presence of foetal components of the placenta prevents the ergot effect from being expressed.

Prevention of ECO interference with pregnancy by (foetal) placental grafts

Assuming it is the presence of foetal placenta that determines ergot inhibition after L_6, then if foetal placenta is made available to pregnant rats before the normal time, it should inhibit the ECO interruption of pregnancy. Rats were selected on L_6 of pregnancy, i.e. before the existence of placentae, and maternal or foetal components of (donor) placentae were introduced into a subcutaneous site. Two hours later ECO was administered.

Placental material for grafting was obtained from L_{14} pregnant donors. The donors were killed, placentae were removed from the uterine wall and separated

in foetal or maternal components, and prepared for grafting into a subcutaneous abdominal pouch. Each of the L_6 pregnant host animals received either four maternal or three foetal 'placentae'. Since complete separation of maternal and foetal elements of the placenta is not possible, experimentally induced deciduomal tissue was obtained from L_8 pseudopregnant rats (whose uteri were traumatized on L_4) in approximately the same quantity as the placental pieces, and grafted into L_6 hosts.

In all ten of the L_6 pregnant animals, bearing foetal placental grafts, pregnancy was maintained normally, thus preventing ECO action. In the series bearing maternal placentae, ECO interruption of pregnancy was inhibited in five out of five females. Of six L_6 pregnant rats which received grafts of deciduomata and were treated with ECO 2 hr later, all had interrupted pregnancies accompanied by onset of oestrus.

Prevention of ECO interference with pregnancy by placental extracts

These experiments were done to test whether the activity (or active principle) of in-situ foetal placentae or the grafts which prevented ECO action, was extractable. The term 'placental extract' is applied loosely, since the extract was made from whole implants. The extract was prepared by killing L_9 pregnant donors, removing their conceptuses and homogenizing these in about 0·2 ml saline/conceptus. The homogenate was centrifuged and the supernatant injected subcutaneously, 0·5 ml/animal, containing the equivalent of 2·7 L_9 implants. The foetus at this early stage is difficult to separate from the whole implant, but, since it is only a very small part and since we have shown that the foetus is not responsible for the phenomenon under discussion, its presence in the extract seems of little importance.

The extract was administered 2 hr before ECO injection to nine rats on L_6 of pregnancy. In five rats, pregnancy was maintained, in four it was interrupted. Onset of oestrus was observed after ECO in all cases of effective interruption, and in one case which, however, maintained pregnancy.

DISCUSSION

By the 7th day *post coitum*, a sharp decline in sensitivity of the pregnant rat to ergot interference with gestation takes place, as observed by Shelesnyak (1955) for ergotoxine and confirmed in the present study for ergocornine (ECO).

We have extended the study to an investigation of the basis for the cessation of the period of susceptibility of the pregnant rat to the action of ECO. In addition to demarcating the period of sensitivity to ECO, we found that raising the dose of the alkaloid does not overcome this decline in susceptibility of the pregnant rat. No such time limit for ECO action is observed in pseudopregnancy. The presence of deciduomata—a tissue closely analogous to maternal placenta —does not give protection against ECO action. Maternal and foetal components of the placenta, left *in situ* after removal of the embryo, prevent ECO action from being expressed. Subcutaneous implants of placental tissue confer protection on early pregnant rats, but grafts of artificial decidual tissue do not.

And finally, saline extracts of whole conceptuses, though less effective than placental tissue, conferred protection against ECO action.

Indications, suggesting a central target of ECO, have been proposed (Zeilmaker & Carlsen, 1962; Varavudhi, 1965); the mechanism of action of ECO on gestation seems to involve a depression of pituitary luteotrophic activity.

Luteotrophic activity may also be abolished by performing hypophysectomy. Pencharz & Long (1933) have shown that hypophysectomy before the 11th day is incompatible with maintenance of gestation and results in abortion. Prolactin is the only anterior pituitary hormone capable of sustaining pregnancy in such hypophysectomized rats (Desclin, 1953; Evans, Simpson & Lyons, 1941; Cutuly, 1941; Selye, Collip & Thomson, 1934).

Astwood & Greep (1938), Averill, Ray & Lyons (1950) and Cannivenc & Mayer (1953) have demonstrated that during the course of gestation the placenta becomes a major source of luteotrophic activity (reviewed by Amoroso, 1960).

If the failure of ECO to interfere with pregnancy by L_7 is to be correlated with the beginning of placental luteotrophic activity by that time, then there is an obvious discrepancy with the findings obtained by hypophysectomy. However, Alloiteau (1957) has suggested that the placenta acquires a luteotrophic function as early as Day 6 *post coitum*. The placental gonadotrophic stimulation at that time is not adequate to ensure sufficient ovarian progesterone secretion to maintain pregnancy in the absence of the adenohypophysis. Nevertheless, it can protect the corpora lutea from regression by secreting enough luteotrophin to keep the corpora lutea sensitive to reactivation by exogenous LTH or by endogenous placental luteotrophin after the 11th day (Alloiteau, 1957).

The observation that placental luteotrophic activity is discernible this early is in accord with the finding that resistance of pregnant rats to ECO interference with gestation begins around L_7. If it is assumed that ECO does not interfere with placental luteotrophic activity, these data are consistent.

The proof that the foetal elements of the placenta were responsible for the failure of ECO action was arrived at from experiments which revealed that neither deciduomata (typifying maternal placenta) nor the presence of the embryo itself, interfered with ECO. In addition, direct evidence of the involvement of placental material in abolishing the effectiveness of ECO was that placental tissue from late pregnant donors protected the early pregnant (L_6) host from ECO. Furthermore, saline extracts of early (L_9) conceptuses, which included placental tissue, acted as did the placental tissue grafts.

Thus a humoral factor, extracted from the placenta, was capable of maintaining gestation following ECO. On the basis of these considerations, this humoral factor can be equated with placental luteotrophin, which has been considered as originating from foetal elements of the placenta. The luteotrophic activity of the maternal placental tissue may be due to incomplete separation from foetal elements, since 'pure' maternal placenta (= experimentally produced deciduoma) did not exhibit any luteotrophic activity.

The ECO-treated pregnant rat, with its selectively disturbed endocrine function, offers a more sensitive test animal for estimation of luteotrophic properties of placental or other tissues than the hypophysectomized rat. Removal of the

hypophysis affects more endocrine factors than is needed for assessment of luteotrophic activity. In fact the pharmacological approach allowed earlier determination of luteotrophic substance in the placenta than did the use of hypophysectomized rats.

The appearance of cornified type vaginal smears in animals whose pregnancies were maintained is by no means unusual after ergot reversal. Inhibition of ergotoxine action by exogenous prolactin (Shelesnyak, 1958), was associated in 43% of cases with an oestrous vaginal smear in otherwise normally maintained pregnancies.

These data give added support to the concept that ECO acts to suppress pituitary LTH, without modifying the capacity of the ovary or, specifically, of the corpora lutea, to respond to trophic stimulation. As soon as the placenta has matured sufficiently to produce enough of its luteotrophic substance, the ECO activity is inhibited drastically and pregnancy is maintained. The precise mechanism of pituitary LTH suppression by ECO is as yet undefined.

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