J. Reprod. Fert. (1969) **18,** 81–88

INTERRUPTION OF EARLY PREGNANCY IN MICE BY ORAL ADMINISTRATION OF AGROCLAVINE AND SCLEROTIA OF *CLAVICEPS FUSIFORMIS* (LOVELESS)

P. G. MANTLE

Biochemistry Department, Imperial College, London

(*Received* 15*th February* 1968, *revised* 13*th August* 1968)

Summary. Pregnant mice fed on a diet containing agroclavine (250 μg/day) during the 2 to 3 days before nidation failed to implant but returned to oestrus within a few days. Eight weeks of agroclavine treatment did not impair the subsequent fertility of virgin mice. Female mice fed on the agroclavine diet conceived normally, and alkaloid treatment during the first 2 days of pregnancy or after implantation did not affect the pregnancy. Agroclavine did not affect pregnancy when injected subcutaneously. Ergotoxine, ergosine and lysergic acid α-hydroxyethylamide were also found to interrupt pregnancy when administered in the diet.

INTRODUCTION

The clavine alkaloids, produced by several *Claviceps* species, have, relative to the related ergot alkaloids derived from lysergic acid, received little attention as physiologically active substances. The only published reports on the pharmacology of the clavine alkaloids have been by Yui & Takeo (1958, 1962, 1964) but, although studies have been made within the pharmaceutical industry, there is no evidence that these substances are regarded as having any useful pharmacological properties relative to some lysergic acid-type ergot alkaloids (Hofmann, 1961).

The effect on early pregnancy of the related group of alkaloids derived from lysergic acid has been extensively studied by Shelesnyak and co-workers (Carlsen, Zeilmaker & Shelesnyak, 1961; Kraicer & Shelesnyak, 1965; Varavudhi, Lobel & Shelesnyak, 1966).

Recently, agroclavine has been shown to inhibit mammary gland development and lactation in mice (Mantle, 1968). This was a new pharmacological action for a clavine alkaloid, and these investigations have now been extended to determine the effect of agroclavine on conception and early pregnancy in mice.

This paper describes an implantation block by oral administration of agroclavine at a non-toxic dosage. This is also a new pharmacological action for a clavine alkaloid, and a similar action on the rat has been confirmed recently by Dr J. A. Edwardson (personal communication).

MATERIALS

Experimental animals

Mature albino mice, strain BS/VS, were used throughout. Animals were fed routinely on pellets of Thompson's rat diet and, during treatment periods, were fed on milled meal of the same constitution with which was homogenized either milled ergot sclerotia or a solution of agroclavine in 3% tartaric acid. In preparation for experimental use, mated animals were inspected in the morning for the presence of vaginal plugs, and the day on which a vaginal plug was observed was regarded as Day 1 of pregnancy or pseudopregnancy.

Ergot sclerotia

Sclerotia of *Claviceps fusiformis* were kindly supplied by the Director, Station Agricole de Saria, Koudougou, Haute Volta. After cleaning to remove adherent floral parts, the total alkaloid content, expressed as agroclavine, was found to be 0·3%. The major alkaloidal components were agroclavine and elymoclavine and the minor components were penniclavine, setoclavine and chanoclavine.

Alkaloid

The alkaloids used and their formulation for incorporation into the food or for subcutaneous injection are summarized in Table 1.

RESULTS

Effect of a 2% ergot diet on fertility

Treated and control groups of virgin female mice were each caged together with a proved male and fed respectively 2% ergot sclerotia and untreated diets (Table 2). Day 1 of pregnancy was estimated by extrapolation from the date of subsequent parturition on the basis of a 20-day gestation. Thus, although one treated replicate conceived on the last day of the treatment period, none of this group actually implanted during treatment. When the remaining treated replicates became pregnant, there was no evidence that the earlier ergot treatment had any lasting effect on fertility. This preliminary experiment indicated that there had been a significant effect on the fertility of the treated group. This could best be explained by an effect on a post-fertilization aspect of pregnancy.

Effect of agroclavine on early pregnancy

Twenty female mice having vaginal plugs were divided into two groups. One group was fed on a diet slightly in excess of requirements (6 g/mouse/day), containing 5 mg agroclavine %, while the other group received an equal quantity of untreated meal. Treatment was started on the day on which vaginal plugs were found and continued until Day 7, when both groups were transferred to the normal diet in unlimited amounts. Eighty per cent of the control group were later found to be pregnant, while no pregnancies occurred in the treated group. It was concluded that agroclavine treatment (slightly less than 300 μg/day) had effected a termination of pregnancy sometime during the first 6 days.

TABLE 1

SOURCE, PURITY AND FORMULATION OF EXPERIMENTAL ERGOT ALKALOIDS

Alkaloid	*Source*	*Purity*	*Formulation*	
			Incorporation in food	*Injection*
Agroclavine	Fermentation of *C. fusiformis*	Nearly pure (Trace of setoclavine)	Dissolved in excess 3% tartaric acid and incorporated at < 3 ml%	Dissolved in slightly excess 0·1 N-HCl. pH adjusted to 6·0 with saturated NaOH*
Ergosine	Sclerotia of *C. purpurea*	Isomeric mixture of ergosine and ergosinine (60:40)		
Lysergic acid α-hydroxy ethylamide	Fermentation of *C. paspali*	Pure		—
Ergotoxine ethanesulphonate B.P. (mainly ergocornine)	Sclerotia of *C. purpurea*	Pure	Dissolved in minimum ethanol, diluted with water and incorporated at <3 ml%	—

*The vehicle alone was found to have no detectable pharmacological action.

The optimal effective period of agroclavine treatment

A preliminary experiment in which mated female mice were fed 5 g of a 5-mg agroclavine % diet for a single day only during the following 6 days did not give conclusive results. Thus, a 2-day period was selected for further experiments at the same dose rate, summarized in Table 3.

Implantation begins between Days 4 and 5, and agroclavine treatment during the first 2 days of pregnancy or after implantation did not affect the development of a normal pregnancy. The most effective period of treatment by the oral

TABLE 2

EFFECT OF MATING VIRGIN FEMALE MICE WHILE FEEDING A 2% ERGOT DIET

	Replicates ♀	♂	*Fed daily*	*Acceptability of food*	*Duration of treatment (days)*	*Replicates conceiving during treatment period*	*Replicates conceiving after treatment period*	*Replicates implanting during treatment period*
Treated	5	1	42 g 2% ergot meal	Poor on first day; thereafter increasing to equal control group by 5th day	20*	1 (Day 20)	4 (Days 2, 9, 10, 12)	0
Control	5	1	42 g untreated meal	Good from first day	20*	5 (Days 1, 5, 10, 15)	0	5

* Thereafter fed normal diet.

TABLE 3

INCIDENCE OF PREGNANCY IN MICE FOLLOWING AGROCLAVINE TREATMENT DURING A SERIES OF 2-DAY PERIODS AFTER MATING

Treatment period	*Agroclavine/ day (μg)*	*Replicates*	*% pregnant*
Days 1 and 2	250	11	73
Days 2 and 3	250	15	30
Days 3 and 4	250	9	0
Days 5 and 6	250	11	0
Days 6 and 7	250	12	42
Days 7 and 8	250	6	83

route was during the 2 to 3 days before implantation, but there was evidence that pregnancy had been terminated in some replicates treated either side of the optimum period. This may have been due to minor variations in their stage of pregnancy derived from the variable time of coitus during the night preceding the finding of a vaginal plug.

Effective dose range of agroclavine

An experiment to determine the minimal effective daily dose of agroclavine required to prevent pregnancy is summarized in Table 4. A dose of 200 μg agroclavine presented daily in the food on Days 3 and 4 of pregnancy (approxi-

mately 165 μg actual dose ingested) affected pregnancy in some replicates, but a daily intake of 250 μg agroclavine was found to terminate pregnancy without inducing any signs of toxicity.

In a further experiment, a total of only 350 μg agroclavine given over the same 2-day period was also effective in terminating pregnancy and this figure was probably close to the minimal effective dose.

Effect on subsequent fertility of terminating pregnancy by agroclavine treatment

Twenty-two mice of known conception date were caged with the male with which they had mated and together fed on a normal diet for 2 days after mating. On Days 3, 4 and 5 of pregnancy the females were fed on the agroclavine diet separately in a small cage within a large cage housing the male, which was fed on a normal diet. This ensured that the female ate all the treated diet, and the mutual contact eliminated any possible chance of a temporary complete separation resulting either in a retarded oestral pattern or in the male subsequently acting as a strange male and inducing abortion in the female. From

TABLE 4

EFFECT OF THREE DOSAGE LEVELS OF AGROCLAVINE ON THE 3- TO 5-DAY STAGE OF PREGNANCY

*Food given on Days 3 and 4** (*g*)	*Agroclavine*		*Replicates with vaginal plugs*	*Replicates pregnant after treatment*
	Given/day (*μg*)	*Estimated mean daily intake* (*μg*)		
6	300	250	5	0
6	200	165	5	2
6	100	85	6	5

* All replicates returned to normal diet on Day 5.

Day 6 onwards the pairs were together fed a normal diet. In this modified experiment, the alkaloid treatment terminated all pregnancies but all twenty-two replicates were found to have mated again 7 to 11 days after the initial mating. Twenty-one of these developed a normal pregnancy, the remaining replicate being pseudopregnant though conceiving 9 days later.

It was notable that half of the replicates which re-mated did so 7 days after the first mating. It is suggested that, for the first pregnancy to have been terminated and a normal oestral pattern to have been established so soon, the most likely effect of the agroclavine diet had been to prevent implantation. Bruce (1960) demonstrated a similar pattern of blocked pregnancy and return to oestrus at about the 7th day resulting from the effect of a strange male during the pre-nidation period, and this has been conclusively established as involving a failure of the blastocysts to implant.

Effect of agroclavine on mating and conception

Ten female mice, which had all previously had one normal pregnancy, were fed on the agroclavine diet (5 g/day) for 5 days before introducing a male, and

examined thereafter daily for the presence of vaginal plugs while both male and female continued to be fed together on the agroclavine diet (15 g/day). When a vaginal plug was found the female was removed to a separate cage and fed a normal diet. Vaginal plugs were observed in six replicates within 11 days of introducing the male, the remainder mating within 27 days. Six out of the ten matings resulted in a normal pregnancy, indicating that the agroclavine treatment given up to Day 1 of pregnancy had not prevented the development of an oestral cycle, nor had it prevented ovulation, mating or subsequent pregnancy. This was in spite of an alkaloid dose sufficient to terminate pregnancy when administered during the 3- to 5-day period of pregnancy.

The experiment was repeated on the four replicates which, though known to have mated, were only pseudopregnant. All these replicates mated within 4 days of introducing the male and developed a normal pregnancy.

Taking the two experiments together, 72% of the observed vaginal plugs resulted in a pregnancy, which was within the normal limits for this colony of mice, and it was concluded that the agroclavine treatment had had no significant effect on the process of conception.

Effect of prolonged agroclavine treatment on subsequent fertility

Ten mature virgin mice were fed in one group for 8 weeks on a diet containing 5 mg agroclavine %, each replicate consuming daily approximately 5 g meal (250 μg agroclavine). There was no evidence of any general adverse effect of this treatment. At the end of the treatment period the females were caged individually with a male, five replicates being fed on a normal diet, the others continuing on the agroclavine diet (15 g/pair) for a further 10 days after which they were transferred to a normal diet. All five replicates which had been transferred to a normal diet when males were introduced, mated within 2 days, four developing normal pregnancies. The other replicates continuing for 10 days on the agroclavine diet also mated during this period but only established a successful pregnancy after returning to a normal diet.

Effect of subcutaneous injection of agroclavine on early pregnancy

A single subcutaneous injection of 250 μg agroclavine had an almost immediate pronounced central stimulatory effect lasting about 4 hr but did not appear significantly to interrupt pregnancy when administered on Day 2, 3, 4 or 5. It was also ineffective when given over a period of up to 4 consecutive days during the pre-nidation period (Table 5).

Comparative effect of feeding agroclavine and other ergot alkaloids on Days 3, 4 *and* 5 *of pregnancy*

Interruption of pregnancy followed the feeding of diets containing agroclavine, ergotoxine, ergosine or lysergic acid α-hydroxyethylamide (Table 6).

DISCUSSION

The purpose of these studies has been to determine whether oral administration of the sclerotia of *C. fusiformis* or their principal alkaloidal component, agro-

clavine, had any effect on conception or early pregnancy. The results indicate clearly that pregnancy can be blocked effectively by feeding a diet containing agroclavine at a non-toxic dosage during the 2 or 3 days before implantation. It has been demonstrated for the first time that the oral route is effective not only for agroclavine, but also for some peptide lysergyl derivatives, in blocking implantation. The group of ergot alkaloids known to have this property has been extended to include not only a simple lysergyl derivative but also a clavine alkaloid; a notable feature of the clavine group being that they have only weak uterotonic and vasopressor actions relative to the classical ergot alkaloids.

TABLE 5

EFFECT OF SUBCUTANEOUS INJECTION OF AGROCLAVINE ON EARLY PREGNANCY

Injected 250 μg agroclavine	*Replicates*	*Pregnant*
Day 2	3	2
Day 3	2	2
Day 4	4	2
Day 5	4	2
Days 4 and 5	7	6
Days 3, 4 and 5	7	3
Days 2, 3, 4 and 5	8	5

TABLE 6

EFFECT ON EARLY PREGNANCY OF FEEDING DIETS CONTAINING VARIOUS ERGOT ALKALOIDS

Alkaloid	*Daily dose* (in 5 g meal) (μg)*	*Replicates*	*Pregnant*
Agroclavine	250	6	0
Ergosine/ergosinine (60:40)	250	5	0
Ergosine/ergosinine (60:40)	500	5	0
Ergotoxine ethanesulphonate	250	6	1
Lysergic acid α-hydroxyethylamide	250	6	0

* Fed on Days 3, 4 and 5.

Kraicer & Shelesnyak (1965) proposed a relationship between structure and function among four ergot alkaloids which blocked pregnancy at varying doses. Although the present studies were not extended to make a critical comparison with these alkaloids, it is clear that both agroclavine and D-lysergic acid α-hydroxyethylamide are similarly potent by the oral route but do not share any particular biodynamic structural features.

Surprisingly, pregnancy was unaffected by subcutaneous injection of agroclavine at a dosage which, if administered in the food, would have blocked pregnancy, and this observation may warrant further investigation. It is possible that an effect on pregnancy could have been obtained by injecting a

larger dose of agroclavine. However, this would have involved approaching a lethal dose (LD_{50} was found to be approximately 35 mg/kg), thus introducing an over-riding factor. Injected agroclavine (250 μg) elicited a pronounced central stimulation, but the animals returned to normal within 4 hr, suggesting elimination of the alkaloid during this period. Further, the rapid recovery of mice to an efficient lactation on ceasing an agroclavine diet (Mantle, 1968) also suggested that the alkaloid was rapidly eliminated. Thus it is possible that injected animals were alkaloid-free for about 20 hr each day and agroclavine may be effective in blocking pregnancy only when a blood level, even though quite a low one, is maintained for 2 or 3 days. Investigations into the mode of action of agroclavine are in progress (Edwardson, personal communication) but it appears that it may not act by triggering a sequence of responses as has been proposed for ergocornine (Varavudhi *et al.*, 1966).

ACKNOWLEDGMENTS

I am grateful to Mr R. A. L. Batt for giving the subcutaneous injections, to Mr R. E. Hales and Miss J. F. Capper for technical assistance and to The Wellcome Foundation Ltd for the supply of ergosine and ergotoxine ethanesulphonate B.P.

REFERENCES

Bruce, H. M. (1960) A block to pregnancy in the mouse caused by proximity of strange males. *J. Reprod. Fert.* **1,** 96.

Carlsen, R. A., Zeilmaker, G. H. & Shelesnyak, M. C. (1961) Termination of early (pre-nidation) pregnancy in the mouse by single injection of ergocornine methanesulphonate. *J. Reprod. Fert.* **2,** 369.

Hofmann, A. (1961) Recent developments in ergot alkaloids. *Australas. J. Pharm.* **42,** 7.

Kraicer, P. F. & Shelesnyak, M. C. (1965) Studies on the mechanism of nidation. XIII. The relationship between chemical structure and biodynamic activity of certain ergot alkaloids. *J. Reprod. Fert.* **10,** 221.

Mantle, P. G. (1968) Inhibition of lactation in mice following feeding with ergot sclerotia. [*Claviceps fusiformis* (Loveless)] from the bulrush millet [*Pennisetum typhoides* (Staph and Hubbard)] and an alkaloid component. *Proc. R. Soc.* B, **170,** 423.

Varavudhi, P., Lobel, B. L. & Shelesnyak, M. C. (1966) Studies on the mechanism of nidation. XXIII. Effect of ergocornine in pregnant rats during experimentally induced delayed nidation. *J. Endocr.* **34,** 425.

Yui, T. & Takeo, Y. (1958) Neuropharmacological studies on a new series of ergot alkaloids. *Jap. J. Pharmac.* **7,** 157.

Yui, T. & Takeo, Y. (1962) Pharmacological studies on clavine-type alkaloids. *Folia pharmac. jap.* **58,** 386.

Yui, T. & Takeo, Y. (1964) Increase in anti-5HT activity by 1-methylation of clavine-type ergot alkaloids. *Jap. J. Pharmac.* **14,** 107.