MECHANISM OF POSTCOITAL CONTRACEPTION WITH A COMBINATION OF STEROIDS AND THE CENTRAL M-CHOLINOLYTIC BENACTYZINE

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Blocking central muscarinic (M-) cholinergic systems with benactyzine facilitates the manifestation of the contraceptive effect of estrogen norsteroids in early pregnancy in rats. This effect is connected with lowering of the gonadotropin level and a change in the ratio between follicle-stimulating and luteinizing hormones, interfering with implantation of the fertilized ovum in the endometrium. The rate of transport of the ovum along the fallopian tube and development of the decidual reaction are also changed. The change in rate of movement of the ovum is not accompanied by any disturbance of fertilization. The substances tested showed no cytotoxic action. The use of mestranol and ethinylestradiol in conjunction with norethinodrel and benactyzine may prove promising as a method of short-term contraception in the postcoital period.

KEY WORDS: postcoital contraception; tubal transport; estrogens; progestins; benactyzine.

The mechanism of postcoital contraception has been inadequately studied [4, 5, 7, 8]. It has been shown [2] that estrogen–norsteroid preparations can exert a contraceptive action without inhibiting ovulation and that this effect is potentiated by neurotropic agents.

In this investigation the contraceptive action of Soviet steroids – mestranol, ethinylestradiol, norethynodrel – and the central muscarinic (M-) cholinolytic benactyzine and also of a combination of ethinylestradiol with norethisterone acetate (the preparation Non-Ovlon, marketed by Jenapharm, East Germany) was studied. In all the experiments the ratio of estrogen to progestagen was 1:20.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature female rats with a normal estrous cycle. The moment of conception was determined from the presence of spermatozoa in the vaginal smear. The substances were administered during the first 3 days after conception by gastric tube in the following doses: ethinylestradiol 0.025 mg/kg, mestranol 0.05 mg/kg, norethynodrel 0.5-1 mg/kg, norethisterone acetate 0.5 mg/kg, benactyzine 5 mg/kg. Altogether 3 series of experiments were carried out on 210 rats.

In series I, to assess the contraceptive activity of the preparations, the number of corpora lutea and implantation sites was counted on the 8th day at laparotomy and the number of corpora lutea and fetuses was counted on the 20th day. In series II the state of the fertilized ova was studied. The animals were decapitated after receiving the preparations for 3 days; the ova were flushed from the oviducts and investigated in the MBS-1 phase-contrast microscope. To assess the state of the embryos they were vitally

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TABLE 1. Comparative Analysis of Postcoital Contraceptive Action of Substances Tested and Their Effect on Development of the Ovum in Rats in Early Pregnancy (72-90 h)

Preparations and daily doses (in mg/kg)	Mean number of corpora lutea per rat	Mean number of normal fertilized ova flushed out	No. of rats with ova flushed out in rel. to No. of rats in exp.	No. of fetuses on 20th day per rat	Contraceptive activity (in %)
Control	12	7	10/11	10	3
Norethynodrel – 1 Mestranol – 0.05	11	7	2/5 $P<0,05$	6	57 $P<0,01$
Norethynodrel – 1 Mestranol – 0.05 Benactyzine – 5	11 M	6	5/11 $P<0,05$	—	100 $P<0,001$
Norethynodrel – 0.5 Ethinylestradiol – 0.025	11	6	3/3	7	60 $P<0,05$
Norethynodrel – 0.5 Ethinylestradiol – 0.025 Benactyzine – 5	11	7	4/6	4	80 $P<0,01$
Norethisterone acetate – 0.5 Ethinylestradiol – 0.025	11	4	4/10 $P<0,05$	7	20
Norethisterone acetate – 0.5 Ethinylestradiol – 0.025 Benactyzine – 5	12	4	4/10 $P<0,05$	8	40 $P<0,05$

stained with neutral red. In series III the content of luteinizing hormone (LH) was determined by Parlav's method and the content of follicle-stimulating hormone (FSH) by Brown's method [3]. The ovaries and uterus were investigated histologically on the 3rd and 6th days after fertilization.

EXPERIMENTAL RESULTS

Administration of mestranol (0.05 mg/kg) with norethynodrel (1 mg/kg) prevented the development of pregnancy in more than half of the rats, and if given in conjunction with benactyzine, they prevented pregnancy in 100% of cases (Table 1). Replacement of mestranol by ethinylestradiol increased the contraceptive activity. Consequently, ethinylestradiol is a more active estrogen, in accordance with data in the literature [6].

Benactyzine potentiated the effect of ethinylestradiol with norethynodrel: It prevented the development of pregnancy in 80% of cases ($P < 0.01$). If pregnancy continued, the number of implanted embryos was reduced to 4 (from 10 in the control). A combination of norethisterone acetate with ethinylestradiol prevented pregnancy in only 40% of cases ($P < 0.05$), and then only if the two substances were given together with benactyzine. The number of rats receiving steroids from whose oviducts ova were flushed out was much smaller than in the control, but cleavage of the zygotes took place normally in the experimental rats, and the embryos, as in the control, were at the 2-4-blastomere stage (Fig. 1). Since a decrease in the number of animals in which fertilized ova were found by the 3rd-4th day was manifested equally after administration of the steroids alone and steroids combined with benactyzine, this suggests that the effect was dependent on the combination of steroids. The number of embryos flushed out of the oviduct per rat was usually 6-7 in the control and experimental series (compared with 11 corpora lutea). Their number was a little smaller when norethynodrel was replaced by norethisterone acetate. Norethisterone acetate probably led to a more marked change in the rate of passage of the ovum along the oviduct. Analysis of the results of the experiments with this substance shows that the embryos reached the uterus when its internal medium was favorable for implantation, and for that reason 7-8 fetuses developed as far as the 20th day of pregnancy. If benactyzine was given in combination with norethynodrel and estrogens the embryos did not develop or there were fewer of them. Considering the role of cholinergic systems in the central regulation of reproductive function at the hypothalamic-pituitary level [1], potentiation of the contraceptive effect of the steroids by benactyzine can conjecturally be attributed to a change in neurohormonal responses.

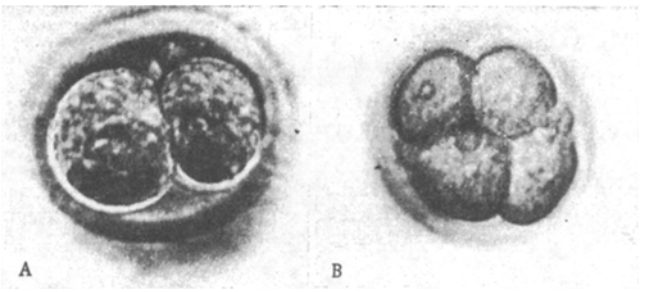

Fig. 1. Fertilized ova flushed out of rats' oviducts 72 h after administration of norethynodrel (1 mg/kg) with mestranol (0.05 mg/kg) and benactyzine (5 mg/kg) for 3 days: A) at 2-blastomere stage; B) at 4-blastomere stage; 100 ×; phase contrast.

TABLE 2. Effect of Substances Administered (3 days, internally) on Concentration of LH and FSH in Plasma of Rats in Phase of Estrus*

Preparation and its dose (in mg/kg)	FSH (in mg/100 ml plas.)	LH (in μg/100 ml plas.)	Ratio LH (in μg)/ FSH (in mg)
Control	28	11	1 : 2,5
Norethynodrel – 1 Mestranol – 0.05	25	2,29†	1 : 10,9
Norethynodrel – 1 Mestranol – 0.05 Benactyzine – 5	12†	0,7†	1 : 17
Norethisterone acetate – 0.5 Ethinylestradiol – 0.025 Benactyzine – 5	7,5†	2,3†	1 : 3,26

*All rats were in the phase of estrus during the 3 days of administration of the preparations; rats in the phase of estrus were accordingly used as the control.
†$P < 0.05$.

Substances possessing contraceptive activity in fact lowered the LH and FSH level in the plasma (Table 2), but the decrease in the FSH concentration was particularly marked after a combination of steroids with benactyzine.

It can be concluded from these observations that blocking the central M-cholinergic systems by benactyzine potentiates the contraceptive effect of estrogen norsteroids in the early stages of pregnancy. The predominant factor in the mechanism of action of these substances is evidently their effect on the hypothalamic-pituitary centers. Lowering the gonadotropin level combined with a change in the FSH/LH ratio probably creates an unfavorable background for tubal transport and implantation of the ovum in the endometrium. Evidence in support of the latter is given by a reduction in decidual-like changes in the endometrium of the experimental animals. The fertilization process itself is undisturbed by the steroids and they have no cytotoxic action.

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