MECHANISM OF THE SYNERGIC ACTION OF ESTROGEN-NORSTEROID COMBINATION AND CERTAIN NEUROTROPIC AGENTS ON REPRODUCTIVE FUNCTION

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Experiments on rabbits showed that the effect on reproduction and the contraceptive action of the combined estrogen-norsteroid hormone preparation infecundin are potentiated by blocking of central cholinergic synapses and by the action of the neurotropic drug ethimizole. Blocking of nicotine-like cholinergic systems by adiphenine and the action of ethimizole potentiate the antiovulatory effect of infecundin. Benactyzine, which blocks muscarine-like cholinergic systems, facilitates the exhibition of other aspects of the mechanism of action of the estrogen-norsteroid combination, and a contraceptive effect is observed even if the infecundin is given during the first 3 days after fertilization.

Estrogen-progestogen combinations of steroids such as mestranol in a dose of 0.1 mg with the 19-norsteroid derivative norethynodrel in a dose of 2.5 mg (such as the product infecundin, approved for use by the Soviet Health Service, and enovid E, used abroad) affect pituitary gonadotropic activity and the reproductive function. When taken internally in certain schemes of dosage they prevent pregnancy in women [4, 9, 10], but the mechanism of the contraceptive action of infecundin and of other estrogen-norsteroid combinations is complex and requires further investigation [4, 10, 18].

Work in the writers' laboratory [5, 6] and elsewhere [3, 7] has demonstrated that the gonadotropin level can be changed by the action of neurotropic nonsteroid agents whose pharmacological properties have been described in detail by Anichkov et al. [1, 2]. For instance, muscarine-like cholinolytics inhibit the luteinizing and follicle-stimulating activity of the pituitary; adiphenine, which affects the central nicotine-like cholinergic synapses, and the original Soviet neurotropic agent ethimizole, act mainly on the luteinizing function.

In the investigation described below the action of ethimizole, benactyzine, adiphenine, and infecundin on the ovulatory function of the ovaries and on the post-coital effect was studied.

EXPERIMENTAL METHOD

Two series of experiments were carried out on 88 sexually mature rabbits weighing 3-3.5 kg. In series I the animals received infecundin, neurotropic agents, or infecundin together with neurotropic agents for 6 days. Fertilization took place in the stage of heat assessed as 4-5 points [11] after 3 days of injection of the drugs. The presence of ovulation was verified by diagnostic laparotomy 24 h after coitus. At repeated laparotomy on the 8th and 29th day the number of corpora lutea, follicles, and embryos was counted. In series II the test drugs were given after fertilization during the first 3 days of pregnancy. All

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TABLE 1. Action of Tested Substances on Reproductive Function in Rabbits

Substance tested and dose (in mg/kg)	Fertilization (number of rabbits)	Ovulation (number of rabbits)	Pregnancy (number of rabbits)	Number of embryos	Number of corpora lutea	Weight of organ (in mg/kg) M ± m*: uterus	Weight of organ (in mg/kg) M ± m*: ovaries	No. of expts.
Control . .	12	12	12	8±0.2	9±0.4	1 370±141	82±6	11
Infecundin 0.5† . .	12	11	10	7±0.3	9±0.2	2 256±110	83±7	5
P						<0.01		
Adiphenine 10,0	22	22	18	8	9	1 338±157	80±3	7
Infecundin 0.5+ Adiphenine 10.0	7	6	3	3	7	1 670±200	69±5	5
P			<0.01	<0.01	<0.05			
Benactyzine 3.0	8	8	7	10±1	10	1 482±157	94±8	8
Infecundin 0.5 + Benactyzine 3.0	5	5	1	9	10	2 386±302	77±5	5
P			<0.01			<0.02		
Ethimizole 10.0	10	8	6	8±0.2	9±0.7	1 210±123	70±5	7
P			<0.05					
Infecundin 0.5+ Ethimizole 10.0	7	4	0	0	5±0.4	1 794±152	85±7	5
P		<0.05	<0.01	<0.01	<0.05			

*Weight of organs determined in unmated animals receiving test substances internally for 6 days.
†As norethynodrel.

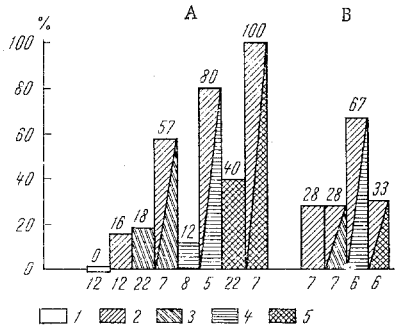

Fig. 1. Contraceptive action of drugs tested: A) coitus 3 days after beginning of 6-day period of administration of the substance; B) substances given during first 3 days of pregnancy. Ordinate, number of nonpregnant rabbits (in percent); abscissa, number of experiments; 1) control; 2) infecundin (0.5 mg/kg); 3) adiphenine (10 mg/kg); 4) benactyzine (3 mg/kg); 5) ethimizole (10 mg/kg).

the substances were given by gastric tube. Infecundin was given as a thin aqueous suspension in a dose of 0.5 mg/kg (as norethynodrel); the 2% aqueous solutions of the neurotropic drugs were given in doses depending on their pharmacological activity: the muscarine-like cholinolytic benactyzine – 3 mg/kg, adiphenine and ethimizole – 10 mg/kg body weight. The experimental results were subjected to statistical analysis with a level of significance $P < 0.05$.

EXPERIMENTAL RESULTS

Infecundin, benactyzine, and adiphenine did not prevent the development of pregnancy (Fig. 1A), but combined administration of infecundin and the cholinolytics in these doses had a marked contraceptive effect. Of 5 experiments in which infecundin was given together with benactyzine, pregnancy occurred in only one ($P < 0.01$). Combined administration of infecundin with adiphenine prevented pregnancy in 50% of cases ($P < 0.01$). A combination of infecundin with ethimizole suppressed the reproductive function of all the experimental animals, whereas ethimizole itself prevented pregnancy in 40% of the experiments (Table 1).

In most animals receiving benactyzine, adiphenine, or infecundin, ovulation took place. This suggests that there is another mechanism of action which participates in the effect of infecundin and its combinations with the central cholinolytics studied on the reproductive function. However, ovulation did not take place in 3

of the 7 animals receiving a combination of infecundin with ethimizole, while in the rest, in which ovulation took place, the number of corpora lutea was reduced to 5 compared with 9 in the control. This result was interpreted as an index of the change in ovulatory function, in agreement with data in the literature [15].

The marked increase in the number of mammary glands filled with colostrum in the 3 rabbits of this group compared with the control even in the absence of pregnancy was evidently due to disinhibition of the lactogenic function, and this may also indirectly indicate inhibition of the other gonadotropins [14, 16].

Histological analysis of the ovaries and uterus on the 8th day after fertilization showed the development of corpora lutea and a decidual response of the uterus corresponding to this period in the control [12].

In the rabbits receiving ethimizole, if pregnancy was absent, fibrosis was found in the center of the corpus luteum and cystic changes in the follicles. The decidual response of the uterus was slight in degree. This also suggests that ethimizole, by modifying gonadotropic activity, led to changes in ovarian function. The results described show that the contraceptive action of the combination of infecundin and adiphenine is based on a change in the quality of ovulation. Although ovulation was preserved in 6 of the 7 experiments, the number of ovulating follicles fell to 7 while the number of fetuses fell to 3 ($P < 0.01$). It can accordingly be postulated that the contraceptive action of the combination of infecundin with ethimizole or adiphenine is due to the absence of ovulation or to a decrease in the number of corpora lutea if ovulation remains present, possibly as the result of a change in the luteinizing activity of the pituitary.

When the substances were given during the first 3 days of pregnancy, before implantation, both infecundin alone and infecundin combined with adiphenine or ethimizole prevented the development of pregnancy in only 28-33% of experiments (Fig. 1B). Blocking of muscarine-like cholinergic structures by benactyzine potentiated the contraceptive effect of infecundin by more than twice ($P < 0.01$) under the experimental conditions specified. This is in agreement with data in the literature on the predominantly estrogenic action of contraceptives of the estrogen-norethynodrel group, which prevents the development of pregnancy when administered in the early stages after fertilization [13]. If infecundin was given together with benactyzine the weight of the uterus was increased. In conjunction with published observations [8] this index can also reflect a change in the estrogen balance following administration of estrogen-norsteroid combinations during blocking of central muscarine-like cholinergic systems.

It can be concluded from analysis of these results that blocking of cholinergic systems by adiphenine or the muscarine-like cholinolytic benactyzine facilitates manifestation of the contraceptive effect of the estrogen-norsteroid combined hormonal preparation infecundin. This effect is potentiated to the greatest degree by the action of the neurotropic agent ethimizole, possessing high activity in the region of hypothalamo-hypophyseal centers. Potentiation of the contraceptive action of infecundin by ethimizole and adiphenine is seen most clearly if the substances are given before fertilization, when it is due to a change in the character of ovulation: either its absence or a decrease in the number of ovulating follicles.

Benactyzine, which modifies not only the luteinizing, but also the follicle-stimulating activity of the hypothalamo-hypophyseal centers, facilitates the depression of reproduction by infecundin in the early periods of pregnancy (before implantation) also.

A change in the follicle-stimulating function evidently plays an important role in the mechanism of the postcoital effect of estrogen-norsteroid combinations with high estrogenic activity.

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