Induction of Ovulation with Synthetic Gonadotrophin-Releasing Hormone in Women with Constant Anovulation Induced by Contraceptive Steroids

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Summary

The ovarian response to stimulation with follicle-stimulating hormone/luteinizing hormone-releasing hormone (FSH/LH-RH) was studied in young, healthy, and fertile women with constant iatrogenic anovulation caused by depot medroxyprogesterone acetate or depot chlormadinone acetate injected for contraceptive purposes. Results were compared with those in unstimulated controls. The response was observed directly on the ovaries at laparotomy performed after treatment with FSH/LH-RH. A wedge biopsy provided ovarian tissue for histological and histochemical studies of steroid dehydrogenase activity. Treatment with FSH/LH-RH caused a trophic effect on the ovaries, with evidence of follicular development; ovulation occurred in two out of 16 treated women. Preovulatory mature follicles were found in three others.

Clearly the FSH/LH-RH-induced release of FSH and LH caused follicular growth up to Graafian follicles, mature preovulatory follicles, and ovulation. Mitosis in granulosa and theca cells was also observed. A wide individual variation in gonadal response to hypothalamic FSH/LH-RH was evident, however. Nonetheless, our data support the possibility that treatment with FSH/LH-RH may prove valuable in patients with anovulatory sterility of hypothalamic origin.

Introduction

Numerous reports have documented the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary in animals and humans in response to stimulation with natural or synthetic hypothalamic LH- and FSH-releasing hormone (FSH/LH-RH). (Kastin *et al.*, 1972 a; Schally *et al.*, 1971 a; Schally *et al.*, 1971 b). It has been clearly shown that the pituitary gland releases mostly LH but also FSH after FSH/LH-RH is injected subcutaneously, intramuscularly, or intravenously. The response seems to be dose-related. Endogenous FSH and LH released under these conditions stimulate the gonads, as shown by the steroids secreted. (Schally *et al.*, 1972; Kastin *et al.*, 1972 b; Yen *et al.*, 1972; Zarate *et al.*, 1972). The study of the gonadal response from the viewpoint of ovulation or spermatogenesis, however, is most important for further assessment of the true potential value of FSH/LH-RH in the treatment of sterility and infertility. (Zarate *et al.*, 1972; Zanartu *et al.*, 1974; Akande *et al.*, 1972).

Pituitary gonadotrophic hormones have both a mitotic action, stimulating follicular growth, and a steroidogenic effect. When the mature follicle is ripe LH forces its rupture and the ovum is released. Since the release of FSH and LH and the resulting hormonal changes in the gonads after injection of FSH/LH-RH have been shown in man and other mammals, we felt that it would be interesting to study the follicular growth and the mitotic and ovulatory response at the ovarian level after FSH/LH-RH stimulation. Modifications which resulted from the steroidal response in other indirect indices were also investigated. In carrying out the study we used an experimental model in which constant iatrogenic anovulation existed as a result of the administration of injectable depot medroxyprogesterone acetate (MPA) and depot chlormadinone acetate (CA) for contraceptive purposes. We expected that more information could be obtained about the effect of FSH/LH-RH on human ovaries and ovulation by directly observing both ovaries at a laparotomy performed for elective tubal sterilization. We thought this could be substantiated by histological and histochemical studies of the structures stimulated and by a comparison with findings in untreated controls.

Patients and Methods

Fifty-seven apparently healthy women, aged 20 to 42 years and known to be fertile, participated in the study. They had all asked for tubal sterilization because of high multiparity. In each case both the husband and the wife had signed a request form after an attending physician and social worker had explained the surgical procedure. The whole investigation was considered and approved by a specially appointed ethical committee at the University Maternity Hospital. Each case had to be approved by at least four of the six physicians on the committee. The clinician in charge carefully explained the preoperative studies to each patient and that at operation a wedge biopsy would be made of one ovary and that one oviduct would be removed for morphophysiological studies.

We assumed from their previous pregnancies that all the women had a normally functioning hypothalamic-pituitary-gonadal axis. Constant iatrogenic anovulation as a contraceptive measure was then induced in all of them by the administration of either long-acting depot MPA or depot CA. The MPA was injected intramuscularly in doses of 250 or 300 mg every six months or 150 mg every three months, the CA in doses of 250 mg every two-and-a-half to three months or 150 mg every two months. These progestogens are potent contraceptives. (Zanartu *et al.*, 1970; Zanartu and Onetto, 1974).

The 57 participants were divided into a control group and an experimental group. The *control group* consisted of 41 women who had been on the contraceptive regimen for more than 12 months. They were then examined at laparotomy for tubal sterilization or for therapeutic hysterectomy. The *experimental* group consisted of 16 women who had also followed the contraceptive regimen. While they were still affected by the MPA or CA—as shown by marked progestational modifications in such target organs as vagina, cervix, and endometrium—they were given stimulatory treatment with synthetic hypothalamic FSH/

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LH-RH. The substance used was a decapeptide with the composition and amino-acid sequence described by Schally *et al.* (1971 b, and 1972).

Since the individual or total dose required for ovarian stimulation was not known the experimental group was further divided into four subgroups, each of which received a different total dose of FSH/LH-RH intramuscularly or by intravenous infusion (see table II) to try to find the most effective dosage in terms of follicular growth, maturation, and rupture with corpus luteum development. Total doses of less than 1 mg, 1 to 1·5 mg, 1·5 to 2 mg, and more than 2 mg were administered. The individual intramuscular dose ranged from 0·05 to 0·1 mg injected once or twice a day over a period of seven to 20 days. All intravenous administration was in the form of infusions, lasting five hours or longer, of 0·5 mg in a 500 ml isotonic glucose solution.

Because of reports that intravenous infusions were most effective (Zarate *et al.*, 1972) the first trials were with five- to seven-hour intravenous infusions every second day. Because this proved inconvenient to patients, however, and because it failed to elicit any marked oestrogenic response, we adopted a regimen of one daily intramuscular injection for seven to 20 days followed by a single intravenous infusion once the intramuscular treatment had induced modifications in the cervical and vaginal indices suggesting endogenous oestrogen secretion. Subsequently we increased the number of intramuscular injections to two a day for the same number of days before giving the single intravenous infusion.

Several criteria were used in deciding when to change from intramuscular injections to intravenous infusions. Endogenous progesterone secretion was estimated from 24-hour urinary pregnanediol excretion, which was routinely studied the day before treatment and before laparotomy, using a thin-layer chromatography method (Sulimovici *et al.*, 1965). The endogenous secretion of oestrogens was evaluated indirectly by biological criteria (vaginal cytology and kariopicnotic index, modifications in the physico-chemical properties of cervical mucus, and cervical os opening). An endometrial biopsy was taken the day before laparotomy.

After treatment the patients were explored at laparotomy 12 hours to 11 days after the final dose of FSH/LH-RH. The uterus, oviducts, and both ovaries were examined by direct visual and manual inspection. The presence of bloody fluid in the peritoneal cavity (pouch of Douglas), signs of ovulation, corpus luteum, cystic follicles, follicular cysts, or an apparently mature follicle were carefully recorded. Photographs were taken in all cases. A wedge biopsy was made in the ovary containing the functional structures estimated to be most significant—that is, mature follicle, corpus luteum, cystic follicle, and follicular cyst. The tissue sample was divided into two portions: one for histological study and the other, frozen with dry ice and sectioned at 16 microns thickness in a cryostat at minus 20°C, for investigation of enzyme activity related to delta-5,3-beta-hydroxysteroid dehydrogenase (3-β-OHD) and 20-alpha-hydroxysteroid dehydrogenase (20-α-OHD). The methods we used have been described elsewhere (Zanartu *et al.*, 1970) and also our classification of the follicular apparatus (Zanartu *et al.*, 1970; Zanartu and Onetto, 1974).

The diagnosis of a fully mature, non-ruptured follicle was based on the macroscopic aspect and the histologic evidence of well-developed granulosa cells and internal and external theca cell layers. The presence of numerous mitoses in these cells was considered an important sign of gonadotrophic stimulation. They were counted on tissue sections by a semi-quantitative method and recorded in arbitrary units (0 to 3+).

Results

CONTROL GROUP

At laparotomy the ovaries of women in the control group appeared normal in shape but they were about two-thirds of the normal ovulatory size (table I). They were whitish in colour, had a smooth surface, and contained from one to three cystic follicles and, occasionally, one or two follicular cysts. No evidence of ovulation or corpus luteum was seen on direct inspection or microscopically (Zanartu *et al.*, 1970; Zanartu and Onetto, 1974). Though there were normal-appearing follicles up to the Graafian stage and atretic follicles there was no mitosis nor was enzymatic activity of steroid-converting dehydrogenases (3-β-OHD or 20-α-OHD) ever present.

It was assumed from these findings that the ovaries were in a "resting," anovulatory condition. This was shown by the fact that though follicular growth up to the Graafian stage persisted and there was some luteinization of the theca there was no evidence of steroid-converting dehydrogenase activity.

EXPERIMENTAL GROUP

All patients in the experimental group, whose ovaries had been stimulated by FSH/LH-RH, presented slight to marked evidence of ovarian stimulation in terms of size. The ovaries consisted of a number of cystic follicles. In two patients there was bloody fluid in the peritoneal cavity. A congested uterus, oviducts, and ovaries were found in the presence of a mature follicle or fresh corpus luteum in five women. Biopsy confirmed the existence of fresh haemorrhagic corpus luteum in two patients. In three further patients the biopsy tissue contained luteinized follicles with well-developed theca and granulosa cells and with mitosis (1+ to 2+). In these structures steroid-converting dehydrogenase activity for 3-β-OHD was positive.

The stimulatory effect of FSH/LH-RH manifested itself in mild to moderate signs of oestrogenic changes in the vaginal cytology and cervical gland secretion, with a switch from progestational to oestrogenic characteristics. Endometrial histology, however, was not modified. It was also apparent that the intramuscular injection of synthetic FSH/LH-RH caused definite stimulation in "resting" ovaries with suppressed ovulation in terms of mitotic and steroidogenic FSH- and LH-like effects in those women receiving the optimum dosage (table II).

Discussion

Previous clinical studies have shown that MPA is an effective

TABLE I—*Control Group of Women with Constant Iatrogenic Anovulation caused by Long-acting Injectable Progestogens*

Intramuscular Anovulatory Agent	No. of Patients	Gonadal Findings								Pregnanediol (mg/24 hr)
		Ovaries				Follicular Morphology				
		Average Size	Average No.							
			Cystic Follicles (<0·5 cm)	Corpora Lutea	Follicular Cysts (>0·5 cm)	Luteinized Follicle*	Mitosis		3-β-OHD	
							Granulosa	Theca		
Medroxyprogesterone acetate	27	‡ Normal	2	0	Occasional	Occasional	Nil	Nil	Absent	<1·0
Chlormadinone acetate ..	14	‡ Normal	2	0	Occasional	Occasional	Nil	Nil	Absent	<1·0

*Cystic follicle with luteinized theca.

TABLE II—*Results of Treatment with Synthetic FSH/LH-RH in 16 Women with Constant Iatrogenic Anovulation*

Anovulatory Agent No. of Patients		FSH/LH Treatment				Time between End of FSH/LH Treatment and Laparotomy	Gonadal Findings						Pregnanediol (mg/24 hr)
		Duration (Days)	No. of Injections		Total Dose		Ovaries			Follicular Morphology			
			I.M.	I.V. Infusion							Mitosis†		
MPA	CA		(mg)	(mg)	(mg)		Size	Cystic Follicles	Corpora Lutea R/L Ovary	Luteinized Follicle	Granulosa	Theca	3-β-OHD
		14	8 × 0·05		0·4	15 hr	N*	5		+			Present
3		12	24 × 0·025		0·6	10 days	N	0					
		10	14 × 0·05		0·7	2 days	N			Preovul.	+	+	
		11	12 × 0·05	1 × 0·5	1·1	18 hr	>N	3	0/1	C. Lut.	+		Present
3		14	18 × 0·05	1 × 0·5	1·4	2 days	>N	5		+	+	+	
		10	10 × 0·1	1 × 0·5	1·5	16 hr	N	4		Preovul.	2+	2+	Present
		10	16 × 0·1		1·6	3 days	>N	5		+			
	2	11	16 × 0·1		1·6	2 days	N	5			+	+	Present
		19	16 × 0·05 3 × 0·1	1 × 0·5	1·6	2 days	>N	3		+			
4		19	24 × 0·05	1 × 0·5	1·7	8 days	>N	5			+	+	
		20	26 × 0·05	1 × 0·5	1·8	6 days	N	4		Preovul.	2+	3+	Present
		22	26 × 0·05	1 × 0·5	1·8	2 days	N	4		+		+	
		14	16 × 0·1	1 × 0·5	2·1	15 hr	>N	3	0/1	C. Lut.	2+	2+	Present
3		16	19 × 0·1	1 × 0·5	2·4	2 days	N	4					
		10		5 × 0·5	2·5	7 days	>N	4		+	2+	2+	
	1	12	16 × 0·1	2 × 0·5	2·6	5 days	N	3		+	2+	2+	

*N = Normal.
†Graded on scale 0-3 +. See text.

contraceptive for at least six months when administered in doses of 250 or 300 mg intramuscularly and is effective for at least three months in doses of 150 mg intramuscularly. The period of ovulation inhibition was two months for 150 mg CA intramuscularly and three months for 250 mg CA intramuscularly. At the end of these periods spontaneous ovulation tends to reappear (Zanartu *et al.*, 1970). We therefore assume that it would be easier to induce ovulation towards the end of the period during which the anovulatory agents remained active. Our findings in this admittedly limited study, however, do not confirm this hypothesis. While they confirm the FSH- and LH-releasing effect of the synthetic hypothalamic hormone, there was much individual variation in the response to stimulation. These variations seemed totally unrelated to the anovulatory agent itself or to the time after the last dose of MPA or CA at which the stimulatory agent was given (table III).

Evidently a total dose of FSH/LH-RH of less than 1·0 mg, even if given by slow intravenous infusion, had only a weak ovarian stimulatory effect under our experimental conditions. On the other hand, rather high doses (2·1 to 2·6 mg) were more effective and they did not produce a hyperstimulation syndrome in the four women to whom they were given. In three women we were able to induce preovulatory follicles characterized by well-developed and evenly distributed granulosa and theca cell layers, with mitosis and signs of external theca vascularization, and in two further patients the treatment brought about fresh haemorrhagic corpora lutea.

Under these experimental conditions the ovulation rate was less than 30% even when the preovulatory follicles were counted. We are now trying to determine whether prolonged stimulation with FSH/LH-RH would induce more ovulations. Up to five prolonged intravenous infusions of 0·5 mg FSH/LH-RH produced only a preovulatory follicle in one patient. Numerous mitoses and granulosa cells were present. The optimum stimulation was apparently produced by giving small intramuscular injections repeated every 12 hours until signs of follicular maturation presented and then a booster dose of 0·5 mg by intravenous infusion.

These preliminary observations suggest that repeated small-dose stimulation is more effective than larger single doses. Earlier investigations also suggest that prolonged intravenous infusions may be as effective as small intramuscular doses repeated every six or eight hours daily. These findings point to a prolonged and continuous FSH/LH-RH stimulation for a period of 10 or more days as a possible means of stimulating follicular growth and increasing the ovulation rate. Our findings suggest that a long-acting depot preparation of FSH/LH-RH would be more convenient for therapeutic use.

The fact that treatment with FSH/LH-RH induced ovulation and development of the corpus luteum in some of our patients supports the view that it may prove valuable in patients with anovulatory sterility of hypothalamic origin. We would emphasize, however, that we have not yet found a therapeutic regimen with this agent that consistently gives an ovulation rate similar

TABLE III—*Doses of FSH/LH-RH and Progestogen Contraceptives and Interval between Treatments in 16 Women with Iatrogenic Anovulation*

FSH/LH-RH Treatment		Long-acting Progestogen Contraception			No. of Months between Last Dose of MPA or CA and FSH/LH Treatment	Gonadal Response to FSH/LH Treatment
Total Dose (mg)	Duration of Treatment (Days)	Dose (mg)		Duration of Treatment (Months)		
		MPA	CA			
<1·0:						
0·4	14		150	≥13	2	Luteinized follicle
0·6	12	150		≤12	3	No response
0·7	10	150		≤12	2	Preovulatory follicle
≤1·5:						
1·1	10	250		≥13	6	Corpus luteum
1·4	14	150		≥13	1	Luteinized follicle
1·5	10	150		≤12	2	Preovulatory follicle
≤1·8:						
1·6	10		250	≥13	3	Luteinized follicle
1·6	11		250	≥13	3	Ovaries larger than normal
1·6	19	150		≥13	2	Luteinized follicle
1·7	19	150		≤12	3	Ovaries larger than normal
1·8	20	150		≥13	1	Preovulatory follicle
1·8	22	150		≤12	2	Luteinized follicle
≤2·6:						
2·1	14	150		≤12	3	Corpus luteum
2·4	16		250	≥13	2	Ovaries larger than normal
2·5	10	150		≥13	3	Luteinized follicle
2·6	16	150		≥13	3	Luteinized follicle

to that obtained under the same experimental conditions with human menopausal gonadotrophin and human chorionic gonadotrophin.

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Endoscopic Pancreatography in Management of Relapsing Acute Pancreatitis

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Summary

Endoscopic retrograde cholangiopancreatography (E.R.C.P.) was attempted in 31 patients suffering from repeated attacks of acute pancreatitis. Pancreatograms were obtained in 25 patients. Twelve showed definite "surgical lesions" (obstructions, strictures, or pseudocysts). In at least three patients failure to obtain a pancreatogram was due to obstruction of pancreatic duct close to the papilla. Two patients, both with pseudocysts, developed a mild relapse of pancreatitis after the procedure. Surgical intervention based on the x-ray findings seemed beneficial in the short follow-up period.

Endoscopic pancreatography can be of value in deciding when surgery is advisable for patients with relapsing acute pancreatitis and in determining the operative appreach. It is an advance in the management of a difficult clinical condition.

Introduction

In Britain about one-third of patients who survive an attack of acute pancreatitis have at least one recurrence (Trapnell, 1966). In some of them a previously overlooked contributory factor (such as gall stones or alcoholism) is found which may be remediable. In others, however, there is no apparent explanation for their recurrent acute relapses. These cases pose problems in management—in particular, whether to recommend surgery and what operation. Information about the state of the pancreatic duct and its drainage should be of help. Until recently pancreatography was possible only at laparotomy (Doubilet *et al.*, 1959) after a duodenotomy, which increased the risk of explanatory surgery. Owing to the recent development of fibreoptic duodenoscopes the papilla of Vater can now be cannulated by endoscopy in conscious, sedated patients and contrast medium for radiography injected into the pancreatic ducts and biliary system. After the original Japanese studies (Ogoshi *et al.*, 1970; Oi *et al.*, 1970; Takagi *et al.*, 1970; Kasugai *et al.*, 1972) many encouraging reports of the technique came from Western Europe and North America (Jean-pierre *et al.*, 1971; Blumgart *et al.*, 1972; Classen *et al.*, 1972; Cotton *et al.*, 1972; Cotton, 1972; Vennes and Silvis, 1972). In experienced hands the success rate is about 90%.

Endoscopic retrograde cholangiography has obvious potential in the diagnosis of patients with difficult jaundice (Blumgart *et al.*, 1972) but the clinical role of endoscopic retrograde pancreatography has yet to be defined. This paper reports our use of it in cases of relapsing acute pancreatitis and our experience of its value as a guide to their management.

Patients and Methods

A total of 31 patients were investigated. Five were women (aged 63, 58, 62, 23, and 79 years respectively) and 26 were men (mean age 43 years, range 21-72 years). Two patients came from India, one from Iceland, and the remainder from the United Kingdom and Ireland. Five had had only one major attack of acute pancreatitis but had continued to have recurrent episodes of pain needing further hospital investigation. The remaining patients had had from two to 20 attacks of acute pancreatitis over a period of up to 15 years. Four patients had undergone cholecystectomy for stones and another patient had been suspected of having a gall bladder stone on cholecystography. Fifteen of the patients drank alcohol rarely, if at all, nine drank alcohol every day but not excessively, and seven patients regularly drank more than one litre of beer or three (English) measures of spirits daily. None of the patients had hypercalcaemia and none out of 21 patients tested had any striking abnormality of serum cholesterol or triglycerides. Two patients were being treated with thiazide diuretics. Two patients had had surgery for peptic ulcera-

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