The Effect of Indomethacin on Spontaneous and Human Menopausal Gonadotropin Induced Pressure Changes in the Tissue and Arterial Vascular System from Human Ovaries Perfused in vitro.

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ABSTRACT

Eight human ovaries were perfused in a closed recirculating system with a semisynthetic, hemoglobin-free medium. Arterial and intraovarian pressures were recorded simultaneously. In three experiments spontaneous pressure changes were noticed. In five experiments pressure changes were induced with human menopausal gonadotropin (hMG) (~ 70mIU/ml follicle stimulating hormone (FSH) and 40mIU/ml luteotropic hormone (LH) II. IRP-hMG). Frequency and amplitude of pressure changes in the arterial system and fluctuations in tissue pressure were reduced with indomethacin (20 µg/ml). In one experiment 40 µg/ml indomethacin had been used. In spite of the high dose of indomethacin arterial and tissue pressure changes were not abolished in all experiments. Therefore it is assumed that vascular and tissue activities are not mediated by prostaglandins only.

KEY WORDS

human ovary, in vitro perfusion, arterial pressure, tissue pressure, hMG, indomethacin.

INTRODUCTION

In 1969, Rotcereto and co-workers (1) reported for the first time that the cat ovary was able to contract. These contractions and their inhibition or stimulation by substances, has been shown for numerous animal species (2). The anatomical basis for such contractions, "smooth muscle cells", or "contractile elements"have also been demonstrated by various groups (3,4). As a sign of "muscular activity", changes in electrical potential has been measured in the ovary of an in vitro perfused uterus-tube-ovary unit (5). Furthermore, contractions of ovarian tissue can be provoked by electrical stimula-

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tion (6,7). Most research groups measure these contractions as changes in ovary length, or changes in pressure using an implanted micro-balloon (8) or tissue probe (9, 10). Independent from the pressure oscillation, considerable changes in arterial tonus occurred which, particularly in the preovulatory phase, were more frequent and of higher amplitude than in the luteal phase (11, 9). It has been known, that indomethacin inhibits gonadotropin stimulated ovulation in rats (18) and monkeys (19) and that indomethacin inhibits rabbit ovarian contractility (20). This association between inhibition of ovulation and contractility by indomethacin initiated this study to observe the influence of the substance on human ovaries with a simultaneous recording of tissue and vascular pressures.

MATERIALS AND METHODS

Eight apparently disease-free ovaries form patients ranging in ages from 22-51 ($\bar{x}$ = 42,5) years were used for the perfusion experiments. Two of the older patients already exhibited oligomenorrhea. None of the patients had received hormonal pre-treatment. Two, because of carcinoma of the collum uteri, were pre-operatively irradiated with radium. The amount of radiation on the ovaries during this procedure was about 800 rd. One ovary exhibited a corpus luteum. After their removal, the ovaries were taken from the operating room to the laboratory in ice-cooled perfusion medium, where under aseptic conditions the main artery was canulated and the vascular system was washed free of blood with heparinised medium. The following perfusion medium was used: 137 mmol NaCl; 5,9 mmol KCl; 1,8 mmol $CaCl_2$, 0,49 mmol $MgSO_4$; 1,22 mmol NaH_2PO_4; 20 mmol $NaHCO_3$, 5,45 mmol D-glucose; 25 g/l Albumin, 1 g/l Epicillin. Colateral vessels were tied off. As reported earlier (11, 12), perfusion took place in a closed, recirculating system with a semisynthetic, hemoglobin-free medium at 36.5° C and a nominal flow rate of 2 ml min^{-1} g^{-1} tissue with continuous registration of pH, Po_2, Pco_2 and arterial flow. The arterial perfusion pressure was measured with a pressure transducer (Statham Db 23, Oxnard California) and a manometer MA 83 (Hellige, Freiburg, Germany). The ovarian tissue pressure was measured by a fluid filled probe with multiple circumferential orifices (0.9 mm diameter) inserted into the ovary tangentially about 3 mm from its periphery. The probe was coupled to a second pressure transducer so that arterial and ovarian tissue pressures could be recorded simultaneously. The data were displayed on a multichannel recorder (Rikadenki, Tokyo, Japan). In 2 experiments, instead of the pressure probe, a micro-balloon catheter with a teflon tip and latex membrane (8) was used, but it was less sensitive than the open probe. Indomethacin (Amuno - Sharp & Dohme) was dissolved in 0.1 M NaOH to a concentration of 10 mg/ml. After a control run, 100 µl of this solution was added to 50 ml of perfusate to give a concentration of 20 µg/ml. Since the concentration of indomethacin did not inhibit or reduce pressure changes in all cases, 40 µg/ml in one case was added. The amount of NaOH added as a solvent had no

									INDOMETHACIN					
			ARTERY			OVARY TISSUE			ARTERY			OVARY TISSUE		
No.	Stimulation	Ovary containing	Frequency n/30'		Amplitude mm Hg	Frequency n/30'		Amplitude mm Hg	Frequency n/30'		Amplitude mm Hg	Frequency n/30'		Amplitude mm Hg
1	-	-	40	10% M 90%	6.0 16.8 33.3	33	10% M 90%	0.17 0.36 1.13	5	10% M 90%	9.5 12.3 21.0	0		- - -
2	-	-	31	10% M 90%	1.5 3.8 7.5	16	10% M 90%	0.17 0.46 2.25	8	10% M 90%	2.1 4.2 7.6	0		- - -
3	hMG	-	36	10% M 90%	1.1 1.76 3.2	8	10% M 90%	0.09 0.36 0.5	8	10% M 90%	0.2 1.2 2.7	0		- - -
4	hMG	tertiary follicle	21	10% M 90%	3.3 4.5 10.1	26	10% M 90%	0.04 0.06 0.11	8	10% M 90%	0.4 2.7 6.8	6	10% M 90%	0.04 0.06 0.96
5	hMG	-	24	10% M 90%	21.8 28.9 43.4	0		- - -	17	10% M 90%	10.4 15.4 34.0	0		- - -
6	-	corpus luteum	31	10% M 90%	1.6 2.7 4.8	23	10% M 90%	0.2 0.46 1.02	16	10% M 90%	1.1 1.8 4.5	5	10% M 90%	0.02 0.05 0.7
7	hMG	-	40	10% M 90%	1.9 3.2 4.4	0		- - -	0		- - -	0		- - -
8	hMG	tertiary follicle	13	10% M 90%	1.9 4.7 7.1	36	10% M 90%	0.12 0.4 0.8	0		- - -	0		- - -

Table 1 Influence of indomethacin (20 µg/ml) on frequency and amplitude of arterial and intraovarian pressure changes. Artery: n/30 min ($p<0,01$), 10%- not significant, median- ($p<0,05$), 90%- ($p<0,05$). Ovary tissue: n/30 min ($p<0,05$), 10%- ($p<0,05$), median- ($p<0,05$), 90%- (not significant). *40 µg/ml indomethacin

influence on the final pH or pressure parameters. In 5 experiments we provoked pressure changes by giving hMG (~ 70mIU/ml FSH, ~ 40mIU/ml LH - II. IRP - hMG) (Humegon - Organon). The number of pressure oscillations in 30 min. was counted (frequency). The pressure recordings not normally distributed were digitalised and the 10-percentil, median and 90-percentile of the amplitudes were evaluated for statistical description in the two tailed Wilcoxon test for paired values.

RESULTS

Table 1 summarises the experimantal results. All the 8 perfused ovaries showed oscillations in arterial perfusion pressure and in three of these the oscillations occured without priming with hMG. Six of the ovaries showed tissue oscillations. In all cases the addition of indomethacin significantly reduced oscillations of the arterial perfusion pressure ($p < 0.01$). There was also a reduction of amplitude ($p < 0.05$). A similar reduction of frequency ($p < 0.05$) and amplitude ($p < 0.05$) was seen in the ovarian tissue. After the addition of indomethacin the arterial pressure baseline rose 10-50 mm Hg in three experiments, fell 10-20 mm Hg in two and remained unchanged in three others. The level of the tissue pressure baseline rose 1-2 mm Hg in two experiments, fell in one by about 2 mm Hg, and was unchanged in 5 cases. In some experiments vascular pressure rise caused a short and slight fall in the tissue pressure. During that time a small decrease in arterial flow was recorded. In some instances, tissue contractions were followed by arterial pressure rises. But normally these changes occured independently of each other. Figure 1 shows an example of the simultaneously recorded pressure. Five to 10 min. after the addition of hMG both recordings showed pressure oscillations. After about 20 min. the perfusion pressure changes began to be periodic. Indomethacin (20 µg/ml) inhibited both aspects simultaneously.

DISCUSSION

Ovarian-pressure oscillations provoked by gonadotropins have been described before (13, 14). Prostaglandins which are found in ovarian tissue (15) may be involved in these pressure changes, because the inhibitors of prostaglandin synthesis diminish or abolish the oscillations. The ovarian tissue pressure recordings showed wide variations in amplitude and frequency. Perhaps a single pacemaker site and propagation pathway were not present. The arterial pressure variations however were regular. Tissue contractions seem to be different from those of the arterial wall. Nevertheless prostaglandins play roles in both types of contractions, since they were provoked by $PGF_{2\alpha}$ (9) and blocked by indomethacin. The very high dose of indomethacin demonstrates that prostaglandins

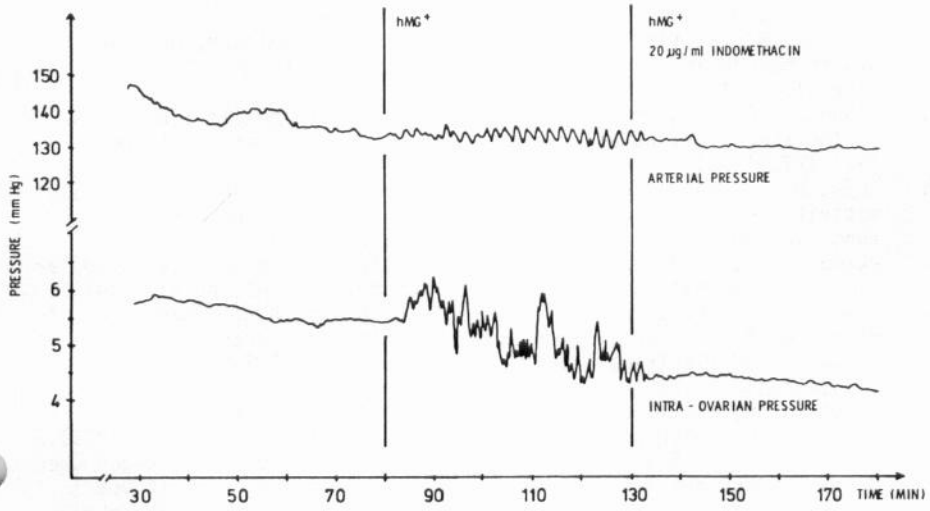

Fig. 1 Influence of indomethacin on the arterial vascular and intra-ovarian pressure of a human ovary with a Graffian follicle after hMG stimulation (70 mIU/ml FSH + 40 mIU/ ml LH - II. IRP -hMG).

are not alone responsible for the noticed pressure changes. This high dose of indomethacin inhibits the 3,5- cAMP- phosphodiesterase by 45% (16) and influences the calcium transport (17). So the nature of intra-ovarian and vascular pressure oscillations seems to be multifarious. The effect of indomethacin on base line tonus was not uniform, so it could be affected in various ways as indicated above and by the possibility that some prostaglandins formed by the tissues are inhibitory (prostacyclin). Besides phosphodiesterase and calcium transport the vascular tone may also be affected by prostacyclin. This study might be an approach in describing the role of tissue and vascular pressure changes and the influence of indomethacin-inhibiting ovulation in hMG treated rats (18) and monkeys (19).

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