The action of vasoconstrictive agents on human tubal arteries

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The aim of this investigation was to compare the response of small arteries of the human tubo–ovarian vasculature to certain vasoactive agents. Ring preparations of the arteries were isolated and mounted in tissue chambers for isometric recording of wall tension. The arteries were exposed to the vasoactive agents adrenalin, prostaglandin F₂α, and two vasopressin analogues, adrenalin, prostaglandin F₂α lysine–vasopressin and triglycyl-lysine–vasopressin all produced powerful vasoconstriction, the greatest efficacy being shown by and lysine–vasopressin. The maximum response occurred after addition of a third compound to a combination of two, irrespective of which combination was used. Adrenalin showed faster contraction velocity than the other agents. The results indicate that the human tubo–ovarian arteries may be constricted by a variety of physiological and pharmacological stimuli, at least partly acting via different effector mechanisms. It is proposed that these vasoconstrictive agents—alone or in combination—may be useful in conjuction with gynaecological endoscopic surgery, e.g. in tubal pregnancy or ovarian cysts. Key words: blood vessels/ectopic pregnancy/oviduct/prostaglandin/vasopressin

Introduction

The complex arrangement of the human tubo–ovarian blood vessels suggests that they serve a specific physiological function. Although not proven to be involved in luteolysis—as in certain other species—this circulation may be important for local endocrine communication within the human genital tract (Bendz et al., 1979). Thus, processes like tubal muscle contraction as well as ciliary activity may be under local hormonal control.

The tubo–ovarian blood flow may also have pathophysiological significance, e.g. in the case of ectopic implantation. More than 95% of such gestations are located in one of the oviducts, and the adnexal circulation is crucial to maintaining such pregnancies.

In a large proportion of tubal implantations, the growth of the trophoblast sooner or later gives rise to rupture of these vessels, which may result in serious intra-abdominal haemorrhage. With the introduction of the new therapeutic procedures for tubal pregnancies, including conservative laparoscopic surgery (Pouly et al., 1986) and local injection therapy (Lindblom et al., 1987; Lang et al., 1990), interest has been directed to the effects of vasoactive agents on these vessels.

In laparoscopic surgery, haemostasis represents an everyday problem and diffuse bleeding may occur in a variety of other interventions, e.g. myomectomy, adhesiolysis or resection of ovarian cysts. Locally acting haemostatic agents may serve an important purpose in this regard, but the information about the effects of different agents on this vasculature is still fragmentary.

The aim of the present study was to compare the response of resistance arteries of the human mesosalpinx to certain vasoconstrictive agents, i.e. the vasopressin analogues lysine–vasopressin and triglycyl-lysine–vasopressin as well as the adrenoceptor agonist adrenalin and prostaglandin F₂α.

Materials and methods

Specimens from the adnexal vasculature were obtained in connection with gynaecological surgery in 16 fertile women (sterilization or hysterectomy). Only macroscopically normal tissue was utilized. A 2 cm long segment of the isthmic portion of the oviduct with the adjoining mesosalpinx was excised and placed in chilled, oxygenated HEPES buffer and transported to the laboratory. Within 30 min mesosalpingal arterial branches (mean external diameter ~0.5 mm) were dissected free from adjacent connective tissue under a stereo microscope (×8–32).

Two arterial segments, both 3.0 mm long according to a microscope scale, were isolated by use of microscissors and mounted in specially designed gallows used for recording transversal wall tension in small blood vessels (Norén et al., 1990). These gallows, which were used instead of the more commonly used metal hooks (e.g. Karlsson et al., 1997), were immersed in a mantled 50 ml tissue chamber filled with HEPES buffer. The temperature was kept at 37°C and the solution was continuously gassed with 100% oxygen, giving a pH of 7.3–7.4. The gallows were connected to a force transducer (Grass FTO3; Grass Instruments, Quincy, MA, USA), and contractile activity was recorded isometrically under a passive tension of 5 mN (Figure 1). Before being exposed to drugs, the specimens were allowed to equilibrate under the applied passive tension for 60 min. The gallows equipment is similar in function to the more commonly used hook system (cf. Karlsson et al., 1997).

The following substances were used: lysine–vasopressin (LVP; Postacton®, Ferring, Malmö, Sweden) triglycyl-lysine–vasopressin (TGLVP; Ferring), adrenalin (Adrenaline, ACO, Stockholm, Sweden) and prostaglandin F₂α (PGF₂α; Prostin®). The Upjohn Company, Kalamazoo, MI, USA). The change in tension induced by a substance

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Figure 1. Relationship between passive tension and maximum active tension ($n = 5$). The standard passive tension chosen for the subsequent experiments, 5 mN, is well within the plateau of the active tension curve. The standard stimulus in this set of experiments was a single administration of adrenalin $10^{-4}$ M.

was registered and the effect of each concentration was expressed as percent of the maximum response induced by the compound.

The concentration of a certain compound that causes 50 and 100% of the maximum effect is designated EC$_{50}$ and EC$_{100}$ respectively. To determine potency, cumulative concentration–effect curves were constructed and EC$_{50}$ values were calculated, assuming that the responses followed the law of mass action. For each compound, the maximal response (efficacy) was determined.

Statistics
In the contractility experiments, an overall analysis of variance with repeated measures was performed. For comparison of changes when a new compound was added, Wilcoxon’s sign rank test for matched pairs was used. A $P$-value of $< 0.05$ was considered significant.

To confirm that the vessels were, in fact, arterial segments of a size corresponding to resistance arteries, a special series of histological experiments was conducted. Segments of blood vessels were collected randomly from 10 patients and fixed in 4% buffered formaldehyde (pH 7.4) for 4 h and rinsed in 10% sucrose in phosphate-buffered saline (PBS)–buffer solution (pH 7.4). After dehydration with increasing concentrations of ethanol and xylene, the specimens were embedded in paraffin. Sections (3 µm thick) were photographed with a Nikon Optiphot microscope using Kodak Tri-X Pan film. A metric microscale was photographed and used to measure the diameter and thickness of the vessels.

The study was approved by the Ethics Committee of the University of Göteborg.

Results
Histological examinations
The thickness of the vessel walls was ~100–150 µm and the mean inner diameter varied from ~250 to 500 µm. The figures were estimated from ~10 measurements of each vessel (Figure 2).

Contractility experiments
None of the specimens tested exhibited any signs of spontaneous contractions during the equilibration period, but all the substances tested induced concentration dependent contractions (Table I). LVP induced a concentration-dependent increase of vessel tone, which was maintained for periods exceeding 60 min (Figure 3). Adrenalin induced a market constrictive response at $10^{-8}$–$10^{-5}$ M (Figure 3). The PGF$_{2\alpha}$ response was less marked than that of LVP and adrenalin. LVP and TGLVP showed similar efficacy but the latter agent had a higher EC$_{50}$ value, i.e. was less potent, and was therefore not included in the further pharmacological experiments (Table I).

The maximum contractile response induced by LVP could be slightly augmented by adrenalin in 10 out of 11 experiments (mean 0.3 ± 0.1 mN) ($P < 0.01$). Conversely, a maximum adrenalin response was enhanced by a maximum concentration of vasopressin in six out of six vessels (mean 6.0 ± 1.1 mN; $P < 0.05$). Furthermore, both LVP and adrenalin were able to enhance the maximal response induced by PGF$_{2\alpha}$ in all cases (LVP: mean 5.6 ± 1.2 nM; adrenalin: mean 4.1 ± 1.4 nM; $P < 0.05$: Figures 4 and 5).

LVP was able to enhance the maximum response of PGF$_{2\alpha}$ + adrenalin (mean 1.1 ± 0.6 mN) in all six cases ($P < 0.05$). Likewise, after a maximum response induced by LVP and PGF$_{2\alpha}$, further augmentation (mean 2.7 ± 1.4 mN) could be achieved by adrenalin in all 11 cases ($P < 0.001$).

In experiments adding a third compound to two others, the response was enhanced in all groups. The augmentation for
**Table I.** Potency and efficacy of, Lysine–vasopressin, Triglycyl-lysine–vasopressin, Adrenalin and Prostaglandin-F$_{2\alpha}$ in segments of human tubo–ovarian arteries

<table>
<thead>
<tr>
<th>Compound</th>
<th>No of specimens</th>
<th>Potency EC$_{50}$ mean ± SEM (mol/l)</th>
<th>Efficacy mean ± SEM (mN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine–vasopressin</td>
<td>5</td>
<td>3.6 ± 0.5 × 10$^{-9}$</td>
<td>8.9 ± 2.4</td>
</tr>
<tr>
<td>Triglycyl-lysine–vasopressin</td>
<td>5</td>
<td>1.1 ± 0.4 × 10$^{-7}$</td>
<td>7.4 ± 1.4</td>
</tr>
<tr>
<td>Adrenalin</td>
<td>8</td>
<td>2.4 ± 0.3 × 10$^{-6}$</td>
<td>9.5 ± 1.7</td>
</tr>
<tr>
<td>Prostaglandin-F$_{2\alpha}$</td>
<td>6</td>
<td>4.5 ± 1.6 × 10$^{-4}$</td>
<td>7.0 ± 1.1</td>
</tr>
</tbody>
</table>

**Figure 3.** Original tracings. Cumulative concentration effect experiment with lysine–vasopressin (LVP), adrenalin (A) and prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$). In all instances, the concentration range from threshold effect to maximal effect is \(~\times 100\).

**Figure 4.** Original tracings. Effects of adding a second and third compound to a preparation where a maximum concentration of a first compound was induced. The second compound was injected when the concentration of the first had reached a plateau. It is clear that the second compound, irrespective of the combination, gives a marked increase of tension (V = lysine–vasopressin, A = adrenalin and F = prostaglandin F$_{2\alpha}$).

Adrenalin as a third compound was 1.7 ± 0.9 mN ($P < 0.001$), for LVP as a third compound 0.9 ± 0.4 mN ($P < 0.01$) and for PGF$_{2\alpha}$ 0.2 ± 0.1 mN ($P > 0.10$, ns).

The results of the analysis of variance showed that the ‘repeat’ factor was significant ($P < 0.001$). Treatment was also significant ($P = 0.015$), i.e. it mattered in what order the drugs were given. The three treatments were regarded as the three orders in which the drugs were given.

The velocity of contraction induced by LVP, adrenalin and PGF$_{2\alpha}$ was studied by registration of muscle activity with extended time scale. Adrenalin constantly had the highest contraction velocity (0.4 ± 0.023 mN/s). LVP and PGF$_{2\alpha}$ exhibited significantly slower contraction velocity. The PGF$_{2\alpha}$ response was of a biphasic character in 56% of instances (Figure 5).

**Figure 5.** Comparison of contraction velocities at supramaximal response ($n = 14$). Note the slow onset of lysine–vasopressin (LVP) (10$^{-6}$ mol/l) and prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) (10$^{-4}$ mol/l) contraction as compared with the rapid development of tension after addition of adrenalin (A) (10$^{-4}$ mol/l). Note also the ‘biphasic’ character of the PGF$_{2\alpha}$ contraction.

**Discussion**

The histological examinations indicated that the vessel preparations studied indeed represent resistance arteries, a category of vessels considered to be primarily involved in the regulation of local blood flow. None of the artery specimens exhibited spontaneous activity, in contrast to the larger branches of the uterine artery (Wilhelmsson et al., 1981).
The present study suggests that the addition of LVP or adrenalin by its vasoconstrictive action on the tubo–ovarian vasculature was the most potent on a molar basis. The potency of PGF\textsubscript{2\alpha} was similar to that of adrenalin. Adrenalin and lysine vasopressin produced similar maximal response, i.e. had similar efficacy, whereas PGF\textsubscript{2\alpha} induced the weakest response of the four agents.

The comparisons of ‘time to maximal response’ showed that adrenalin had the highest contraction velocity. The observed difference in contraction velocity may indicate different modes of action. This is compatible with the finding that the compounds could reciprocally augment one another’s maximal response. The differences in contraction velocity may reflect utilization of different calcium pools intracellularly and/or at the membrane receptor level. Theoretically, a compound with a fast onset (adrenalin) acts preferentially by increasing the calcium influx through receptor-operated channels (Andersson and Högestätt, 1984). Likewise, compounds with slow onset (PGF\textsubscript{2\alpha} and LVP) would reflect the time needed to cross the cell membrane and activate intracellularly stored calcium ions. Although not specifically studied and quantified in the experiment protocol, it was observed that the duration of the response of vasopressin was longer than that of adrenalin or PGF\textsubscript{2\alpha}.

It must be emphasized that our ambition was not to perform a full-scale pharmacological analysis of the action of these agents on the peripheral vasculature. Comparisons with other vascular beds were not performed, since the main objective was to compare the local action of the different vasoconstrictors on these vessels with regard to clearly defined clinical problems.

Local injection of PGF\textsubscript{2\alpha} under laparoscopic control has been shown to be an alternative to surgery in selected cases of ectopic pregnancy (Lindblom et al., 1987, 1990; Paulsson et al., 1995). PGF\textsubscript{2\alpha} exerts an antigonadotropic action on the corpus luteum of pregnancy, increases tubal contractility and causes tubal vasoinconstriction (Hahlin et al., 1987). The mechanisms responsible for the therapeutic effect of PGF\textsubscript{2\alpha} in vivo are, however, far from clear (for reviews, see Lindblom, 1992, 1994). Since luteal function and progesterone concentrations are usually markedly depressed in ectopic pregnancy, the antigonadotropic action of PGF\textsubscript{2\alpha} is probably of little importance in the majority of cases (Hahlin et al., 1991). Furthermore, PGF\textsubscript{2\alpha} and its analogue, 15 methyl PGF\textsubscript{2\alpha}, have little effect on the viability of cultured human trophoblastic cells (Bengtsson et al., 1995). Therefore, it seems probable that the main in-vivo effect of PGF\textsubscript{2\alpha} on ectopic pregnancies is exerted by its vasoconstrictive action on the tubo–ovarian vasculature and perhaps also by its contracting effect on the tubal muscle. The present study suggests that the addition of LVP or adrenalin to PGF\textsubscript{2\alpha} could serve two purposes: (i) the vasoconstriction produced would be more powerful that that of PGF\textsubscript{2\alpha} alone, and the risk of operative and postoperative bleeding would thus be reduced, (ii) the stronger—and probably more long-lasting—effect of the drug combination would ‘trap’ PGF\textsubscript{2\alpha} in the adnexal area and increase the time during which a sufficient concentration of the agent is present in the target tissue.

Another rationale for considering using a combination of two or three compounds—each at a reduced dose—is that the risk of systemic side-effects increases with the dose given. The most prominent of the side-effects of LVP are hypertension, bradycardia and pallor. Adrenalin may cause tachycardia and would, in this respect, counteract the effect of LVP. PGF\textsubscript{2\alpha} may induce coronary arterial constriction but only at high doses accidentally given intravenously. The effect of PGF\textsubscript{2\alpha} on blood pressure is moderate at the doses used here.

Hyperosmolar glucose has been shown to be at least as effective as PGF\textsubscript{2\alpha} for local medical treatment of ectopic pregnancy (Lang et al., 1990). Glucose at high concentrations causes significant inhibition of trophoblast growth in vitro (Bengtsson et al., 1995) but, according to our recent observations, produces insignificant contractile effects on isolated tubo–ovarian arteries at equivalent concentrations (B.Lindblom, unpublished data). Accordingly, a combination of several substances, acting on different target tissues, appears to be of great interest for further development of local medical treatment for ectopic pregnancies (cf. Landström et al., 1998). In addition, the powerful vasoconstriction achieved by combining two or three compounds may be useful in connection with certain other gynaecological operations, e.g. laparoscopic salpingostomy for ectopic pregnancy, treatment of intramural fibroids, haemorrhagic luteal cysts, adnexal adhesiolysis etc.

Theoretically, local injection of vasoconstrictive agents in the adnexal area could induce devastating effects on the ovary with the potential risk of compromising ovarian function acutely or chronically. Although these agents, particularly vasopressin analogues, have been used extensively in gynaecological surgery, there are no indications of such harmful effects. It should be emphasized, however, that to date no studies have addressed this question specifically.

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