Direct effects of the prostaglandins E2 and F2α on progesterone release by the corpus luteum of the marmoset monkey (Callithrix jacchus) studied by in vitro microdialysis

A Fehrenbach², J K Hodges¹ and A Einspanier¹

¹Department of Reproductive Biology, German Primate Centre, Kellnerweg 4, D-37077 Göttingen, Germany
²Centre of Anatomy, Electron Microscopy, University of Göttingen, Kreuzhergring 36, D-37075 Göttingen, Germany

(Requests for offprints should be addressed to A Einspanier, German Primate Centre, Kellnerweg 4, D-37077 Göttingen, Germany)

Abstract

The effects of the prostaglandins (PG) E2 and F2α on progesterone secretion in luteal tissue (32 corpora lutea) explanted from the mid-luteal ovary of the marmoset monkey (n=13) were investigated using an in vitro microdialysis system. Consecutive applications of 1, 10 and 100 µg/ml PGE2 resulted in a significant increase in secretion of progesterone at the maximum dose of 100 µg/ml, which was shown to be the stimulatory dose in both long-period and 20-min pulse (time to collect one fraction) applications. The response varied individually between 1.4- and 3.4-fold above the baseline concentrations. Application of 500 µg/ml PGF2α led to similar hormone responses. In contrast, lower doses of PGF2α (0.5, 5 and 50 µg/ml) resulted in significantly increased levels of secretion of progesterone, to approximately 1.4-fold baseline values, only after the application was terminated (echo effect). Responses were less variable when a short pulse of 20 min duration was applied, instead of long applications of 1–2 h. On the basis of the passage rates measured for tritiated PGF2α, transfer through the dialysis membrane was assumed to be in the range of 1% for both PGs. Ultrastructurally, luteal cells lying in a sheath of five to seven cell layers around the dialysis tubing appeared intact and were interconnected by gap junctions. Vesiculation of the smooth endoplasmic reticulum was more prominent after PG treatment, indicating a stimulation of cellular synthesis/secretory activities that was in accordance with the stimulatory action of both PGs on progesterone release under these in vitro conditions.

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Introduction

Prostaglandins (PGs) are assumed to be important local regulators of luteal function in all eutherian mammals. Production of both luteotrophic and luteolytic PGs and expression of specific PG binding sites have been verified in the corpora lutea of non-primate (reviewed in Auletta & Kelm 1994) and primate species (Challis et al. 1976, Patwardhan & Lanthier 1980, Johnson et al. 1988, Sargent et al. 1988, Houmard & Octobre 1989, Fisch et al. 1994). In women and non-human primates, stimulation of progesterone secretion has been demonstrated for PG E2 (PGE2) in vivo and in vitro (Stouffer et al. 1979, Zelinski-Wooten & Stouffer 1990, Zelinski-Wooten et al. 1990) and has been shown to be mediated by c-AMP (Hamberger et al. 1979, Deneffors et al. 1982, Hahlin et al. 1988, Michael et al. 1993). In contrast, PG F2α (PGF2α) has been shown to reduce the concentration of progesterone released by luteal tissue in vivo (Auletta et al. 1984, Sargent et al. 1988, Auletta & Kelm 1994) and in vitro (Hamberger et al. 1979, Deneffors et al. 1982, Patwardhan & Lanthier 1984, Auletta et al. 1995) via stimulation of protein kinase C (PKC) independently of c-AMP (Endo et al. 1992, Michael et al. 1993).

Treatment with PGE2 was able to prevent PGF2α-induced premature functional luteolysis in the rhesus monkey, but not spontaneous regression of the corpus luteum (Zelinski-Wooten & Stouffer 1990). Therefore, luteal function has been assumed to be regulated by interaction of luteotrophic and luteolytic PGs. On the basis of these results and the observation that the PGE2 : PGF2α synthesis ratio declined from the mid- to the late-luteal phase, induction of luteolysis in primates has been suggested to be actively initiated by endogenous PGF2α (Challis et al. 1976, Balmaceda et al. 1980, Patwardhan & Lanthier 1980, Sargent et al. 1988, Michael et al. 1994).

Evaluation of data relating to the effects of PGF2α on luteal function in primates shows that the method, the concentration used, and the time in the luteal phase when the tissue was obtained are important variables in establishing valid conclusions (Auletta & Flint 1988). In the rhesus monkey, human chorionic gonadotrophin (hCG) was able to override PGF2α-induced luteal regression...
in vivo (Auletta & Keln 1994), although luteal tissue pretreated with PGF$_{2\alpha}$ in vivo exhibited a decreased production of progesterone when stimulated by hCG in vivo (Auletta et al. 1995). In the marmoset, in vivo administration of cloprostenol, a synthetic PGF$_{2\alpha}$-analogue, induced luteolysis during the mid- and late-luteal phases (Summers et al. 1985). Pretreatment with cloprostenol prevented stimulation of progesterone by hCG in vivo, but no antigonadotropic effect was obtained after co-application of both substances (Hearn & Webley 1987). An antigonadotropic action of PGF$_{2\alpha}$ has also been described in cultured human granulosa-luteal cells (Michael & Webley 1991a,b) and cultured slices of human luteal tissue, in which it appeared to be restricted to mid- (Hamberger et al. 1979, Dennefors et al. 1982) or mid-to-late luteal phases (Patwardhan & Lanther 1984). In contrast, PGF$_{2\alpha}$ did not show any antigonadotropic, but some luteolytic, effect in cultures of enzymatically dispersed suspensions or monolayers of luteal cells isolated at the mid-luteal phase in women (Richardson & Mason 1980, Endo et al. 1992), rhesus (Stouffer et al. 1979) and marmoset (Michael et al. 1993) monkeys. Although both luteolytic and luteotrophic effects of PGF$_{2\alpha}$ in luteal tissue/cells and in granulosa luteal cells are suggested to be mediated by activation of PKC (Michael & Webley 1991), this apparent use of the same pathway leading to an opposite hormonal response has not been investigated further.

It has been assumed that the antigonadotropic mechanisms believed to underlie the luteolytic action of PGF$_{2\alpha}$ in vivo require a certain degree of luteal tissue integrity/cell–cell contact, because stimulation of release of progesterone by PGF$_{2\alpha}$ can only be observed in a dispersed cell system (Auletta & Flint 1988, Michael et al. 1994). In contrast, the action of PGE$_2$ is independent of cell–cell contact, consistently leading to stimulation of progesterone release in the different systems used (Michael et al. 1993). We therefore compared these two different substances using an organ perfusion technique, the in vitro microdialysis system (MDS). In contrast to static culture systems, MDS allows immediate changes in hormone responses to be determined and is therefore more sensitive to the concentration and the mode of application of test substances. The method was initially developed for in vivo endocrine studies in freely moving animals (Vaupe et al. 1988, Jarry et al. 1990, Einspanier et al. 1991) and subsequently modified for use in freshly excised pieces of luteal tissue from the cow (Sauerwein et al. 1992, Miyamoto et al. 1993), human (Maas et al. 1992) and marmoset (Einspanier & Hodges 1994). On the basis of ultrastructural investigations of dialysed luteal tissue from the marmoset, the technique was further modified in order to improve the maintenance of cell and tissue integrity (Fehrenbach et al. 1995).

The aim of this study was to compare the direct effects of both PGs on steroidogenesis in marmoset luteal tissue isolated at the mid-luteal phase, in relation to concentration, mode of application and tissue integrity under controlled conditions by using in vitro MDS.

### Materials and Methods

#### Animals

Thirteen adult female common marmoset monkeys (Callithrix jacchus) with normal ovarian cycles were used in this study. The animals were housed in pairs under controlled conditions (24°C, 50–60% humidity, 13 : 11 h light : darkness photoperiod) in the German Primate Centre, Göttingen (Einspanier & Hodges 1994). All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH publication 85–23, revised 1985).

Blood samples (0·2 ml) were collected once or twice weekly by puncture of the vena saphena, and plasma progesterone content was determined in order to monitor ovarian cyclicity. The day of ovulation was defined as that preceding an increase in plasma progesterone concentration above 10 ng/ml (Harlow et al. 1983). Ovarian cycles were controlled by i.m. injection of a luteolytic dose (0·8 μg) of a PGF$_{2\alpha}$ analogue (Estrumate, Pitman-Moore, UK) on day 12 of the luteal phase, which usually lasts 18–20 days (Harlow et al. 1983). Ovulation in the marmoset monkey occurs, on average, 10·7 days after PGF$_{2\alpha}$-induced luteolysis (Summers et al. 1985). In order to ensure that the females did not conceive during the study, they were separated from their male partners on the day of PGF$_{2\alpha}$ application and were returned 6 days after ovulation. This separation did not change normal ovarian cyclicity.

#### Tissue collection

Collection of corpora lutea was carried out between days 8 and 10 of the luteal phase (mid-luteal). The animals were sedated with a mixture of ketamine and xylazine (0·2 ml/animal, i.m.) and narcotised with a halothane–nitrous oxide–oxygen mixture. Laparotomy was performed and tissue removed by luteectomy, a procedure that has previously been reported for the use in the marmoset (Webley et al. 1989). As the marmoset monkey is multiovular (Tardif et al. 1993), two or three corpora lutea were usually present in each cycle and could be excised partially from the ovaries of each female. The tissue was maintained in Dulbecco’s modified Eagle’s medium (DMEM, ICN Biomedicals, Eschwege, Germany) at room temperature for about 20 min until transfer to the perfusion chamber. The medium was supplemented with 4 mM Hepes (Calbiochem, La Jolla, CA, USA), 2 mM glutamine (ICN Biomedicals) and 1%
BSA (Fraction V; Sigma, St Louis, MO, USA) and adjusted to 300 mOsmol (pH 7-4). The animals recovered fully from anaesthesia within 2–4 h after surgery and were returned to their partners 2–3 days later. The animals ovulated between 8 and 11 days after luteectomy, which was in the range of a normal follicular phase. Luteectomy was performed twice in eight females, with an interval of recovery of at least 2 months between each surgery.

In vitro MDS

The in vitro MDS has been described in detail previously (Miyamoto & Schams 1991, Maas et al. 1992, Einspanier & Hodges 1994). The method was further modified for the use with marmoset luteal tissue (Fehrenbach et al. 1995). Briefly, a dialysis tubing (Amicon Diafilter-20 (Amicon, Millipore, Eschborn, Germany); 250 µm outer diameter, molecular mass cut-off 30–50 kDa, 3 mm length) was inserted into a piece of luteal tissue (approx. 4 × 5 × 5 mm³) by means of a fine syringe needle. A drop of fibrin glue (Tissuecol, Immuno, Heidelberg, Germany) was applied at both sides of penetration between tubing and tissue to stabilise and seal the system. The tissue was superfused by DMEM at a flow rate of 60 ml/h. During dialysis, Ringer solution (Braun, Melsungen, Germany) was pumped through the dialysis tubing at a flow rate of 0.5 ml/20 min. The Ringer solution was supplemented with low-density lipoprotein (LDL; Sigma, 0.5 µg/ml) and 1% BSA (Fraction V, Sigma) and was adjusted to pH 6.9–7.2 and 290 mOsmol just before use. After 2 h of preincubation, the dialysate was collected every 20 min. PGs were first dissolved in ethanol, which was diluted with dialysis medium to a final concentration of 1%.

Transfer rates of PGs via the dialysis tubing were determined by using tritiated PGF₂α (2 × 10⁶ c.p.m./ml; Amersham Buchler, Braunschweig, Germany) as tracer substance. As the two PGs used have quite similar molecular weights, we assumed that the transfer rates of PGE₂ and PGF₂α would be of the same magnitude. In order to saturate non-specific binding of the PGs, five membranes were dialysed for 1 h with PGF₂α before fractions were collected every 15 min. The recovery of the tritiated substances was determined by a liquid scintillation counter (1209 Beta-Rack; Pharmacia, Freiburg, Germany).

Hormone assay

The concentration of progesterone in the fractions collected during dialysis was determined by direct enzyme immunoassay, which has previously been described in detail for the use in the marmoset monkey (Hodges et al. 1988). Sensitivity of the assay, defined as the hormone concentration at 90% binding, was 120 pg/ml and the intra- and interassay coefficients of variation calculated from the repeated measurements of quality controls were each below 15%. There was no cross-reactivity between the applied PGs and progesterone antibody in the assay.

Fixation and tissue processing

Immediately after dialysis, the tissue was fixed in 3% paraformaldehyde and 2.5% glutaraldehyde and postfixed in 1% OsO₄, both made up in 0.1 M sodium cacodylate buffer (pH 7.3). Semi-thin sections (1 µm) were stained with a mixture of azur II and methylene blue (1 : 1) according to Richardson et al. (1960). Ultra-thin sections were stained with lead citrate and uranyl acetate. A detailed description of the methods of processing and sectioning used for light and electron microscopy is available elsewhere (Fehrenbach et al. 1995).

Experimental procedure

Dialysis was performed on a total of 32 corpora lutea. Four corpora lutea served as controls (without application of any test substance). The tissue was dialysed for 2 h (preincubation) until baseline secretion values were constant. Depending upon the experimental design, the application of PGs was initiated between 3 and 5 h after the start of dialysis, which was usually restricted to 8 h and did not exceed 11 h.

In order to establish a dose–response relationship between PGE₂ (Serva, Heidelberg, Germany) and progesterone secretion, three corpora lutea were subjected to consecutively applied doses of 1, 10 and 100 µg/ml PGE₂ during a dialysis period of approximately 11 h. The applications lasted for 1 h each and were interrupted by intervals of 2 h of pure Ringer dialysis. In order to investigate the effect of the mode of application, the effective dose of 100 µg/ml PGE₂ was applied in two different schedules: a long-term application for a period of 2 h (three corpora lutea) and a single short-term or pulse application for a period of 20 min (five corpora lutea).

The dose–response relationship between PGF₂α (Serva) and progesterone release was established by single-pulse application mode (20 min) using concentrations of 0.5 (three corpora lutea), 5 (six corpora lutea), 50 (three corpora lutea) and 500 µg/ml PGF₂α (three corpora lutea).

Statistics

All hormone values stated in the text are the means ± s.d. of the grouped data, unless otherwise indicated. The comparisons between the different treatment groups were performed by one-way repeated measures ANOVA and the Student–Newman–Keuls test as a multiple comparison method, provided that the criteria of normal distribution were met (SigmaStat for Windows 1.01). A probability of less than 0.05 was accepted as significant.
Results

System validation

Approximately 1% of the PGF$_2$ applied passed through the wall of the dialysis tubing. This transfer rate remained stable throughout the test period. The total recovery of tritiated PGF$_2$ was around 90%, and approximately 8% of the substance was adsorbed on the dialysis membrane.

Application of PGs

Controls After 2 h of preincubation, baseline release of progesterone remained relatively stable in each corpus luteum (CL) during the following dialysis period (Fig. 1A). There was a statistically significant difference in the amount of progesterone release among the different corpora lutea, with baseline progesterone concentrations of 0.6 ± 0.1, 0.7 ± 0.1, 0.9 ± 0.15 and 1.2 ± 0.14 ng/ml. No relationship was found between the variation in progesterone output by the corpora lutea collected from each animal. Solvent effects caused by ethanol, which was used to dissolve the PGs, could be excluded. No significant changes in progesterone secretion were found when a pulse (20 min) of ethanol (1%) was applied to a piece of luteal tissue while it was being dialysed (Fig. 1B).

Applications of PGE$_2$

Consecutive applications of 1, 10 and 100 µg/ml PGE$_2$ led to a significant increase in progesterone secretion at the maximum dose of 100 µg/ml (Fig. 2). In each CL, hormone release remained increased during the time of application (1 h). The relative increase in baseline progesterone values varied between individual corpora lutea: 1.6-fold in CL1 (baseline 1.6 ± 0.09 ng/ml), 3.4-fold in CL2 (baseline 1.3 ± 0.07 ng/ml) and 1.4-fold in CL3 (baseline 1.1 ± 0.05 ng/ml). There was no significant change in baseline progesterone secretion during the application of 1 and 10 µg/ml PGE$_2$. CL2 was highly responsive to a dose of 100 µg/ml PGE$_2$ (3.0 ng/ml) and showed a measurable but not significant increase in progesterone values with 10 µg/ml PGE$_2$.

Long-duration applications of 100 µg/ml PGE$_2$ for 2 h (Fig. 3A) resulted in a significant and relatively stable increase in progesterone release (CL1, 1.3-fold above baseline of 0.9 ± 0.09 ng/ml; CL2 and CL3, each 2-fold the respective baselines of 0.3 ± 0.05 ng/ml and 0.9 ± 0.1 ng/ml). After application was finished, hormone levels immediately decreased to the initial baseline levels.

Pulse applications (20 min) of 100 µg/ml PGE$_2$ resulted in an immediate and significant increase in progesterone concentrations that was in the range of 2–2.6 times baseline values (Fig. 3B). After application was finished,
hormone values declined to baseline, in the range of the levels before application. No relationship could be established between the level of baseline secretion of progesterone and PG-induced relative increase in progesterone release.

Applications of PGF$_{2\alpha}$ Dose–effect relationships between PGF$_{2\alpha}$ and progesterone after single-pulse applications of 0·5, 5, 50 and 500 µg/ml PGF$_{2\alpha}$ showed two different hormone response profiles (Fig. 4). With 500 µg/ml PGF$_{2\alpha}$, release of progesterone increased immediately during the PG pulse, to values 2–2·3-fold higher than baseline (similar to a pulse of 100 µg/ml PGE$_2$). At the end of the application, the hormone levels returned abruptly to baseline values. In contrast, a different progesterone response was seen with the lower doses, and was characterised by significantly increasing progesterone concentrations, not during the application but in the 1 h thereafter (1·4-fold the initial baseline). This resulted in a greater release of progesterone in the period of dialysis after application compared with the period before the pulse (echo effect). Baseline release of progesterone was stable in each CL, but quite variable among the corpora lutea of the different experimental groups, ranging from 0·4 to 2·1 ng/ml.

Microscopy

After 8 h of dialysis, a sheath of approximately five to seven layers of apparently intact cells surrounded the dialysis membrane (Fig. 5). Tissues treated with PGs did not appear to be histologically different from those of untreated groups (Fig. 6). Ultrastructurally, untreated cells usually displayed tubular cristae in their mitochondria, which were centralised, surrounding a euchromatic nucleus (Fig. 7A). The smooth endoplasmic reticulum (sER) appeared either tubular or vesiculated, depending on the cell type. The cells were interconnected by gap junctions (Fig. 7B), which were maintained in the tissue after treatment with PGs (Fig. 8A). However, in these structurally intact cells surrounding the dialysis tubing, fine structure revealed a more prominent vesiculation of sER than in untreated cells (Fig. 8B). Vesiculation predominated in the perinuclear region when 100 µg/ml PGE$_2$ had been applied for 2 h, and was even more pronounced in cells that had received a 20-min pulse of PGE$_2$ or PGF$_{2\alpha}$, independent of the concentration applied. At the ultrastructural level, no differences in effect could be observed between the two PGs used.

Discussion

In the present study we have used the in vitro MDS in order to characterise the effects of PGE$_2$ and PGF$_{2\alpha}$ on release of progesterone by marmoset luteal tissue collected during the mid-luteal phase. Both PGs stimulated progesterone release, which, in the case of PGE$_2$, confirmed the generally observed luteotrophic action of this substance (Stouffer et al. 1979, Zelinski-Wooten & Stouffer 1990, Zelinski-Wooten et al. 1990). Application of 100 µg/ml PGE$_2$ resulted in an immediate increase in progesterone secretion, which was similar to the mode of action of PGF$_{2\alpha}$ in its highest dose. This was considered to be the maximally effective dose, as higher doses (300 µg/ml), which had been used in a previous pilot study, did not further increase progesterone release. Lower doses of PGE$_2$ did not show any consistent effect on baseline release of progesterone during in vitro MDS. In contrast, stimulation of progesterone by PGF$_{2\alpha}$ revealed two distinct dose–dependent patterns of direct action on luteal tissue: a high–dose response and a low–dose response, described as an echo effect.

Whereas the pattern of low–dose stimulation observed during in vitro MDS is described here for the first time, a concentration of 500 µg/ml has been shown previously to
act similarly on in vitro microdialysed luteal tissue and in luteal tissue from women, causing an immediate increase in progesterone release (Maas et al. 1992). By reducing the time of application to a short pulse of 20 min (time required to collect one fraction), we were able to confirm this pattern of high-dose response, and could further distinguish it from the echo effect observed with the lower doses. In contrast to other in vitro incubation techniques, in vitro MDS makes it possible to detect dose-dependent modulations of the luteal response immediately after application of PGs, by continuous exchange and collection of secretory products. In consequence, in vitro MDS appears to be a more sensitive method, leading to the detection of the initially described echo effect at all low treatment doses. Although in two of the doses tested the number of replicates was low, a similar trend in the change of hormone levels was found. Any effect of ethanol, which was used to dissolve the PGs and was shown to cause slight modulations of progesterone levels at a concentration of 1%, may be considered to be negligible because, with decreasing doses of PGF$_2$ into the dialysate, the ethanol content was also diluted, to a final 0.0001%.

It has to be taken into account that this consistent difference in sensitivity to PGF$_2$ and to PGE$_2$ may have been influenced by the experimental conditions to a certain extent. In a previous study on the ultrastructure of luteal tissue, we found that, after dialysis under control conditions, the tissue appeared to be synthetically activated (Fehrenbach et al. 1995). This was confirmed by translocation of mitochondria and lipid droplets into the perinuclear region and vesiculation of the smooth endoplasmic reticulum (sER) in all tissues after dialysis; both these ultrastructural changes represent characteristics of stimulated cellular synthesis/secretory activities. On the assumption that both PGE$_2$ and mechanical stimuli during dialysis invoked the same cAMP-mediated signal transduction pathway, mechanical activation of hormone synthesis may lead to desensitisation for PGE$_2$. Conversely, the action of PGF$_2$ has been proposed to be mediated by PKC (Abayasekara et al. 1993, Michael et al. 1993), which may be unaffected by the dialysis procedure, allowing PGF$_2$ to stimulate release of progesterone at lower concentrations. Whether this is of biological relevance still awaits elucidation. Equipotent amounts of 1% (transfer...
rate) of the applied doses of 0.5 and 5 µg/ml (but not 500 µg/ml) of PGF_2α had been detected in primate corpora lutea collected at the mid-luteal phase (Patwardhan & Lanthier 1980, Houmard & Ottobre 1989). As PGF2α is quite ubiquitous in tissues and cells, we suggest that, in a CL of the mid-luteal phase, physiologically low concentrations of this molecule should support luteal function rather than counteract basal progesterone production.

To date, no consistent results on a direct luteal action of PGF_2α have been obtained by in vitro studies using static tissue incubation techniques (Patwardhan & Lanthier 1984, Michael & Webley 1993). However, significant stimulation of progesterone release by PGF_2α has been measured in culture systems using dispersed luteal cells (Stouffer et al. 1979, Richardson & Masson 1980, Endo et al. 1992, Michael et al. 1993). It has been hypothesised that the antigonadotrophic mechanisms underlying the luteolytic action of PGF_2α require a degree of tissue integrity/cell-to-cell contact in order to ensure some type of cell-to-cell communication (Auletta & Flint 1988). Tissue integrity was proposed to determine the mode of action of PKC leading either to luteotrophic effects in dispersed cell systems or to luteolytic responses in intact tissue (Michael et al. 1993). This conclusion is not substantiated by studies on dispersed cells, which described a failure of PGF_2α to stimulate basal progesterone production (Richardson & Masson 1980) or that it even reduced an hCG-stimulated progesterone response (Thomas et al. 1978, Hall & Robinson 1979, Stouffer et al. 1979). In addition, granulosa luteal cells, although enzymatically dispersed, were able to respond in a manner similar to that observed in whole-tissue incubations (Michael & Webley 1991a,b). Granulosa luteal cells even luteinise in response to the loss of intercellular contact and, with respect to PGF_2α, finally display functional characteristics similar to those of luteal tissue in vivo. We therefore argue that intercellular communication via cell–cell contact is the decisive factor shifting the response to PGF_2α to a more physiological pattern. In addition, tissue integrity as defined here does not include a functioning microvascular system, which may be the initial site of PGF_2α action in vivo.

To investigate which part of the tissue is influenced by the dialysis procedure, two dialysis tubings were placed side by side in one CL and perfused in parallel with pure Ringer solution and hCG respectively (data unpublished). The effect of hCG remained restricted to the tubing to which it was applied. This was finally confirmed by a study on cellular fine-structure showing that, after 8 h of dialysis, tissue integrity was restricted to a region close to the dialysis tubing comprising five to seven layers of cells (Fehrenbach et al. 1995). The dark staining of these cells in the semi-thin sections was predominantly due to the

Figure 5 Control tissue after 8 h of dialysis without PG treatment. Layers of dark staining cells (arrows) are present next to the dialysis tubing (d). Original magnification × 350.

Figure 6 Marmoset luteal issue treated with a 20-min pulse of 100 µg/ml PGE_2 while being dialysed. A sheath of dark staining cells (arrows) surrounds the dialysis tubing. Original magnification × 350.
Figure 7  (A) Luteal cell after 8 h of dialysis. Mitochondria (M) are centralised, surrounding a euchromatic nucleus (N) and sER appears tubular, with a few vesiculated fields in the perinuclear region (arrow). lp, Lipid droplets; Nu, nucleolus. Original magnification × 12 000. (B) Part of a gap junction (GJ) still present among luteal cells lying close to the tubing after dialysis. Original magnification × 120 000.
Figure 8 (A) Gap junction (GJ) among luteal cells after dialysis including an application of 100 μg/ml PGE₂ for a period of 2 h. Original magnification × 150,000. (B) Luteal cell treated with a 20-min pulse of 5 μg/ml PGF₂α during dialysis. Smooth endoplasmic reticulum (ser) appears vesiculated. M, mitochondrion; N, nucleus; Nu, nucleolus; mvb, multivesicular body. Original magnification × 15,000.
perinuclear aggregation of mitochondria, which appeared as darkly stained granules in light microscopy. Intense staining for the activity of the enzyme 3β-HSD only appeared in this region, indicating that only cells of this part actively participated in microdialysis. Ultrastructurally, these cells were interconnected by gap junctions, which provide the structural basis for paracrine interactions. However, this sheath of apparently intact tissue was surrounded by a larger sheath of cells in deterioration, which itself was surrounded by some layers of intact cells in contact with the medium.

There is some evidence that even small pieces of luteal tissue maintained under in vitro culture conditions for some hours suffer from hypoxia followed by cellular deterioration and tissue lesions. Thus the question arises as to the extent to which whole-tissue preparations can be standardised with respect to the number of cells experimentally involved.

We therefore conclude that each system used produces specific inherent stresses that finally determine the response pattern of the cells. We would also advocate a critical assessment of all techniques used, with special focus on desensitisation by mechanical prestimulation of the cells. We would also advocate a critical assessment of all techniques used, with special focus on desensitisation by mechanical prestimulation of the cells. We would also advocate a critical assessment of all techniques used, with special focus on desensitisation by mechanical prestimulation of the cells.

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