respect to its location in the mitochondrion. Super-dense granules (100-400 A°) which are irregular in shape are often included in the mitochondrial bodies. Endoplasmic reticulum in the cytoplasm is sometimes arranged so that it appears continuous with the outer mitochondrial membrane.

DISCUSSION

The mechanism whereby *Acanthamoeba* mitochondria become cup-shaped or form large dense bodies in their interior during cyst induction is not clear. However, it is well known that aerobic respiration as measured by oxygen consumption decreases rapidly during encystment (Klein and Neff, 1960). In addition, Sobota *et. al.* (1981) reported Ca^{++} and Mg^{++} accumulation in *Acanthamoeba castellanii* under conditions of high cultural tonicity and attributed specific developmental changes in mitochondria and other cellular organelles to calcium binding sites where these ions accumulated. Since large intranuclear microfilaments are also induced in *Acanthamoeba* during preencystment (Tomlinson, 1984), it is tempting to postulate a "Ca^{++} trigger" for *Acanthamoeba* analogous to the role of this ion in vertebrate muscular contraction. Storage of excess calcium in mitochondria of *Acanthamoeba* may alter respiratory mechanisms, induce mitochondrial membrane invagination via local tonicity alteration, and serve in some manner as binding sites for electron dense stains such as those utilized in this study. And how are these changes "reversed" in *Acanthamoeba*? That, too, is not clear at this time. However, the fate of intramitochondrial cups and large electron dense bodies in *Acanthamoeba* are currently under investigation in this laboratory and will be the subject of a future communication.

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EFFECTS OF VARIOUS PROSTAGLANDINS ON OVARIAN FUNCTION IN THE RAT

MARY C. TOMLINSON AND THOMAS T. CHEN
University of Tennessee
Knoxville, Tennessee 37996

ABSTRACT

The hypothesis postulating that prostaglandin $F_2\alpha$ ($PGF_2\alpha$) induces luteolysis by restricting blood flow to the corpus luteum was tested. Immature female rats bearing functional corpora lutea were treated with various vasodilating or vasoconstricting prostaglandins, and levels of progesterone and 20α-dihydroprogesterone in serum and in medium of cultured luteal cells were used as the index of luteal cell function. Although luteal function generally follows the vascular action of prostaglandins (i.e., vasodilators are luteotrophic and vasoconstictors are luteolytic) this logic does not apply to all prostaglandins. These results indicate that a vasoconstricting property does not ensure luteal regression and $PGF_2\alpha$-induced luteolysis is caused by factors in addition to decreased ovarian blood supply.

INTRODUCTION

In the mammalian ovary, the corpus luteum (CL) is formed after ovulation from the follicle which releases the egg. The main function of the corpus luteum is to secrete progesterone which maintains pregnancy by inhibiting uterine contraction. In the absence of conception, however, the CL degenerates which is characterized by a sharp drop in serum progesterone levels and a subsequent rise in the level of its metabolite, 20α-dihydroprogesterone (20α-DHP). This process, known as luteolysis, is caused by one prostaglandin, $PGF_2\alpha$ which is a 20-carbon unsaturated carboxylic acid with a cyclopentane ring. Prostaglandin $F_2\alpha$ has been noted for its potent luteolytic effect in many species including humans (Vijayakuman and Walters, 1983). Evidence for the luteolytic action comes partly from the ability of exogenously administered $PGF_2\alpha$ in regressing the CL and the identification of $PGF_2\alpha$ as the naturally-occurring luteolysin in sheep by Goding et al. (1974). Other evidence includes: Increased length of luteal phase in the rat following injection of indomethacin, which suppresses production of all classes of prostaglandins (Patrono et al., 1976); prolonged estrus cycle in the cow and sheep when passively immunized with $PGF_2\alpha$ antibodies (Fairclough et al., 1981); and morphologic degeneration of the CL by $PGF_2\alpha$ in the guinea pig (Pavvola, 1979).

Other prostaglandins are effective in modifying luteal function. For example, PGE_1, although less potent than $PGF_2\alpha$, is luteolytic in some species, while $PGE_2\alpha$ appears to be luteotrophic in many species. Because of the major physiological effects of prostaglandins is their ability to constrict or dilate blood vessels, a hypothesis has been formulated to explain the underlying mechanism of $PGF_2\alpha$-induced luteolysis. The hypothesis is supported by fragmented evidence showing that some vasodilating prostaglandins are luteotrophic, while vasoconstricting prostaglandins are luteolytic (Milvae and Hansel, 1983). In

general, the PGE's and PGA's are classified as vasodilators, while the PGB's and PGF's are classified as vasoconstrictors. However, no systematic studies are available that definitively test the universality of the "blood flow hypothesis". This study was performed with vasodilators and vasoconstrictors in order to gain insight into the cause of luteal maintenance and regression. The possibility of a prostacyclin (PGI_2) receptor on the ovary, and luteal effects of PGI_2 were also examined separately in this experiment. This report describes the resulting progesterone and 20α-DHP levels, as well as LH receptors on the ovary of rats treated with one of six different prostaglandins.

MATERIALS AND METHODS

Animals

Immature female CD strain rats were obtained from Charles River Laboratories (Wilmington, MA). At 25 days of age, rats were primed subcutaneously with 50 international units (IU) of pregnant mare serum gonadotropin (PMSG), followed by 25 IU of human chorionic gonadotropin (HCG) 56 hours later. The animals were provided with standard rat chow and tap water and were housed in air conditioned quarters with a dark:light cycle of 10:14.

Chemicals

The prostaglandins used in this study, PGE_1, $PGF_1\alpha$, $PGF_2\alpha$, PGA_2, PGB_2, PGI_2, and Lutalyse were purchased from the Upjohn Company. Radiolabeled progesterone (carrier free sodium 125_1, Progesterone-11α-glucuronide-125_1-iodotyramine) and 20α-DHP (1,2,6,7-^{3}H-20α -hydroxy-pregn-4-en-3-one) were purchased from Amersham Corporation (Arlington Heights, IL). All other chemicals of reagent grade were purchased from Sigma or Fisher.

Prostaglandin treatment and sample collection

In both experiments, prostaglandins were administered as 250μg prostaglandin as described under the respective experimental descriptions. Control animals received vehicle only. At selected times, animals were sacrificed by decapitation and trunk blood was collected for determination of serum levels of progesterone and 20α-DHP. Ovaries were removed, cleaned of fat, and either placed in Eagle's MEM/Ham's F12 (E/H) tissue culture medium (Gibco, Grand Island, NY) for ovarian cell culture or frozen on dry ice and stored at −70°C until LH binding studies.

Culture of ovarian cells

Ovaries from each treatment group were weighed, pooled, and minced with a sterile razor blade. Minced tissue was dissociated with Type V crude collagenase (2000 units/gm tissue added) in E/H medium. The dissociation was carried out at 37°C in a Dubnoff shaking incubator for one half hour period, and two subsequent one hour periods, each using fresh collagenase. After dissociation was complete, the final cell pellet was washed three times in E/H media and was resuspended in E/H media with 10% fetal bovine serum, 0.2% gentamycin and 2% fungizone (Gibco, Grand Island, NY). One and one half milliliters of cell suspension were plated into each sterile 35mm Falcon culture dish containing a sterile glass slip to facilitate cell attachment. Cells were cultured at 37°C in a Forma Scientific water-jacketed incubator with humidified 95% air and 5% CO_2. Culture medium was changed every 24 hours and frozen for steroid determination 48 hours after initial incubation for each time period. Cells were examined daily using an Olympus IMT inverted microscope.

Steroid level determination in serum and cell media

Serum levels of progesterone and 20α-DHP were determined by radioimmunoassay after extraction with hexane. Steroid levels in culture medium were assayed without hexane extraction. The procedures for radioimmunoassay for progesterone were described by Gibori et al. (1977), and radioimmunoassay for 20α DHP were described by Resko et al. (1974). Dr. Gordon D. Niswender of Colorado State University supplied the antibodies used in all experiments. The hormone production by each culture dish was corrected for total DNA content for each dish.

Quantification of LH receptors, protein, and DNA content

LH binding capacity in ovaries from control and prostaglandin-treated animals was determined by a radioreceptor assay using ^{125}I-HCG (CR 121) with specific activity approximately 30 mCi/mg. Specific HCG binding was determined by subtracting binding in the presence of 100 fold excess unlabeled HCG from total binding. The LH binding capacity was corrected for ovarian protein content which was determined by the method of Lowry (1951). Total DNA in each culture dish was determined by the method of Burton (1956).

Experiment #1

Forty-eight PMSG/hCG-primed rats were divided into four groups, control rats (vehicle only) and rats receiving 250μg of the following prostaglandins: PGE_1, $PGF_1\alpha$, and $PGF_2\alpha$. Treatments were initiated seven days after PMSG priming. Ovaries and trunk blood were collected at three hours, one day, and three days after treatment to determine the effect of each prostaglandin on blood levels of hormones and luteal cell function.

Experiment #2

Sixty-four PMSC/hCG-primed rats were treated with the following: Vehicle only, PGA_2, PGB_2, and $PGF_2\alpha$; $PGF_2\alpha$ was given as Lutalyse (Upjohn, Kalamazoo, MI). Treatments were, as before, initiated seven days after priming. Animals were killed at three hours, one day, and three days after treatment, except for four control animals, which were killed at the time of vehicle injection. Ovaries and trunk blood were collected from all animals for steroid assay, LH receptor quantification, and DNA and protein determination.

Experiment #3

Twenty PMSG/hCG-primed rats were injected with 250mg prostacyclin (PGI_2) seven days after priming. Five animals were killed at each of the following time periods: 30 minutes, 80 minutes, 5 hours, and 2 days post treatment. Twenty-four control rats (vehicle only) were killed as above, except that four controls were killed immediately after vehicle injection. Ovaries and trunk blood were collected as in previous experiments for steroid quantification, LH receptor quantification, and DNA and protein determination.

Results

Experiment #1

Prostaglandin $F_2\alpha$ induced a 60% reduction in serum progesterone levels within three hours after treatment. Although the levels are not different at 24 hours post injection but after three days the progesterone levels are less than 50% that of controls. On the other hand, PGE_1 had no effect on serum progesterone levels throughout the experimental period, while $PGF_1\alpha$ resulted in an approximately 60% increase in progesterone three days after treatment (Table 1).

All three prostaglandins caused significant increases in serum levels of 20α-DHP on Day 1 with $PGF_2\alpha$ exhibiting the most dramatic effect (2.5 fold increase). However, by Day 3, the 20α-DHP levels had returned to control values in all treatment groups except for the $PGF_2\alpha$ group (Table 2).

Two days after culture, progesterone production by luteal cells removed three hours after prostaglandin injection was essentially the same regardless of treatment (Table 3). When ovaries were removed three days after treatment, progesterone production by cultured ovarian cells from $PGF_2\alpha$ injected rats was slightly less than controls. The production of 20α-dihydroprogesterone production by cultured luteal cells from $PGF_2\alpha$-treated rats was significantly higher than controls and other treatment groups when ovaries were removed three hours post-injection. However, when ovaries were removed three days later, 20α-DHP levels in medium from all three prostaglandin-treated groups were substantially lower than controls (Table 4). Total DNA content of ovarian cultures was not different among the treatment groups except for $PGF_2\alpha$-treated rats whose ovaries were removed at three hours. Therefore, no correction of the steroid production by cultured cells for DNA was necessary.

TABLE 1. *Serum Progesteron (% of Control)*

Treatment		3h		3d	
PGE_1	D	108		116	
$PGF_{1\alpha}$	D	74.6		165*	
$PGF_2\alpha$	C	39.6*		47*	
PGA_2	D	72		175*	
PGB_2	C	83		228*	
			(5 hours)		(2 days)
PGI_2	D		128		179*

*statistically significant ($p < 0.05$)
D = vasodilator
C = vasoconstrictor

TABLE 2. *Serum 20α dihydroprogesterone (% of Control)*

Treatment	1d		3d	
PGE_1	135		100	
$PGF_{1\alpha}$	164		87	
$PGF_2\alpha$	250*		52.5	
PGA_2	31		85	
PGB_2	63		95	
		(5 hours)		(2 days)
PGI_2		177		120

*statistically significant ($p < 0.05$)
D = vasodilator
C = vasoconstrictor

TABLE 3. *Progesteron in Media (% of Control) 24 hour culture period*

Treatment	3h		3d	
PGE_1	110		95	
$PGF_{1\alpha}$	71.4		73.5	
$PGF_2\alpha$	104		60	
PGA_2	47.6		---	
PGB_2	54.9		---	
PGI_2	76	(5 hours)	179	(2 days)

TABLE 4. *20α-dihydroprogesterone in Media (% of Control) 24 hour culture period*

Treatment	3h		3d	
PGE_1	138		26	
$PGF_{1\alpha}$	85		12	
$PGF_2\alpha$	247		53	
PGA_2	60			
PGB_2	64			
PGI_2	67	(5 hours)	129	(2 days)

Experiment #2

At three hours after treatment, PGA_2 and PGB_2 had below control levels of progesterone; but these levels rose to over 100% of control values by Day 3 in both groups.

Serum levels of 20α-DHP were lower than control values one day after PGA_2 and PGB_2 treatment, but they returned to control levels within three days. Levels of progesterone in media was stimulated in cell cultures all three days of culture in cells from PGA_2-treated animals when the cells were stimulated with 8-Bromoadenosine 3′,5′-cyclic monophosphate (cAMP) or isoproterenol (epinephrine). Progesterone levels otherwise were not significantly affected by treatment group or cell stimulation.

Cells from animals treated with PGB_2 and $PGF_2\alpha$ produced higher than control levels of 20α-DHP on Days 1 and 3 of culture. Cells from $PGF_2\alpha$-treated animals produced more than control levels of 20α-DHP when not stimulated or when stimulated with cAMP or isoproterenol. Also, cells from PGB_2-treated animals which were stimulated with cAMP or isoproterenol produced more 20α-DHP than unstimulated cells or any control or PGA_2 cells.

Experiment #3

Animals who were treated with PGI_2 had a steady increase in serum progesterone levels beginning 80 minutes after treatment (data not shown) and remained high (179%) through Day 2. Serum levels of 20α-DHP remained near control levels until 5 hours post treatment when they rose by 77% and remained slightly higher (20%) than controls through Day 2.

In cultured ovarian cells, levels of progesterone from PGI_2 treated animals were significantly higher than from control animals until five hours after treatment. Progesterone levels were again higher in cells from PGI_2 treated animals than from controls on Day 2 after treatment.

Levels of 20α-DHP fell steadily through the five hour time period, and rose by Day 2 in cultures from PGI_2

treated animals. Cultures from control animals produced increasing amounts of 20α-DHP until Day 2, when levels fell.

DISCUSSION

The purpose of this study was to examine whether restriction of blood supply to the corpus luteum is the primary mechanism of $PGF_2\alpha$-induced luteolysis. Our approach was to examine whether the effects of various vasoactive prostaglandins were in agreement with the effects expected due to the vascular action. Although the luteolytic effect of $PGF_2\alpha$ was undisputable, our study indicates no clear pattern of luteal regression or maintenance related strictly to vasoconstriction or vasodilation. The results indicate that when luteal cells are exposed to vasodilators and vasoconstrictors *in vivo*, the resulting maintenance or degeneration of the corpus luteum does not necessarily correspond to the expected result based solely on knowledge of vascular action of the treatment. The results question the importance of vascular action in luteal function. These will be addressed in the following discussion.

We elected to use 250μg for all prostaglandin doses, because at this dose, the luteolytic effect of $PGF_2\alpha$ is well established. Based on our knowledge about the short half-life of all classes of prostaglandins and the similarity of chemical structure and metabolic pathway (site of metabolism), this dose should be sufficient to exert effects, if any, although a dose-response study for some of the prostaglandins would be in order.

This study produced some interesting results. PGB_2 treated animals produced high amounts of progesterone in the second experiment. This prostaglandin is a vasoconstrictor, and progesterone levels would be expected to be low by Day 1 and remain low through Day 3 under these treatments. We did not expect high levels of progesterone at any time points. Another interesting result of this experiment was that treatment with PGA_2, a vasodilator, resulted in lower progesterone levels than PGB_2 on Day 3. These results indicate that luteal and vascular actions of a particular prostaglandin are not always logically connected.

It is important to examine the observed effects in light of several studies on the effects of prostaglandins. The blood flow hypothesis has met with conflicting evidence. In the transplanted sheep ovary, inhibition of progesterone following $PGF_2\alpha$ treatment is not necessarily associated with decreased blood flow (Labhsetwar, 1974). These results, as well as the results of our experiment suggest that these prostaglandins are involved in complex hormonal interaction when exerting their effects on the corpus luteum.

The simple blood flow hypthesis may not be sufficient in the face of other evidence about $PGF_2\alpha$. For example, the effects of $PGF_2\alpha$ are not limited to vasculature. Increased amounts of progesterone have been observed *in vitro* following treatment of cell cultures with $PGF_2\alpha$ (Labhsetwer, 1975). There is more than one explanation for this occurrence. It is possible that the $PGF_2\alpha$ contains PGE_2, which is known to stimulate progesterone production through formation of cAMP. Also, $PGF_2\alpha$ has been converted to PGE_2 under laboratory conditions by Garcia et al (1977). Whether this type of reaction can take place in the body or *in vitro* is unresolved.

There have been many proposals as to how prostaglandins affect luteal functioning. Possible explanations include desensitization by release of luteolytic levels of pituitary luteinizing hormone (LH) (Rothchild, 1981), change in viscosity of luteal membranes (Carlson et al., 1982), or uncoupling of the receptor-cyclase complex in the luteal cell (Jordan, 1981). Direct action of prostaglandins on steroidogenesis is also a distinct possibillity. The increase in pituitary LH following $PGF_2\alpha$ injection indicates another possible mode of action, since LH is luteolytic in pseudopregnant rabbits (Labhsetwar, 1974). Clearly, vascular insult is not the only possible mechanism for luteolysis or luteal maintenance.

Other evidence questioning the role of vascular insult in luteolysis culminated from research by Labhsetwar (1975). First, some researchers failed to detect decreased ovarian blood flow following $PGF_2\alpha$ treatment in sheep, hamsters, and rats. Futhermore, PGE_1 is a vasodilator, yet some have found it to be luteolytic in rats and hamsters. Finally, transplantation of luteal tissue (which involves transient withdrawal of blood flow) results in survival of the tissue in sheep. This particular experiment would be analagous to mechanically performing what $PGF_2\alpha$ has been believed to do chemically, i.e. severely interrupting blood supply to the corpus luteum. All these investigations imply some other cause of luteolysis than vasoconstriction.

The mechanism of the antifertility effect of PGE_1 was studied by Chatterjee (Chatterjee, 1975). It was shown that prolactin can, to some extent, reverse the antifertility effects of PGE_1. In our experiment, PGE_1 had no significant effect on serum progesterone production, though it is classified as a vasodilator (Kadowitz, et al., 1976). It is possible that vascular effects are masked due to a short half-life or that PGE_1's antifertility effects are unrelated to regulation of blood flow. In prostaglandins in general, the vascular effects could possibly initiate some effects on the corpus luteum, but the regression or maintenance could conceivably be due to ovarian hormones themselves. To illustrate, $PGF_2\alpha$ is a vasoconstrictor, but more specifically, it is a venoconstrictor, acting on the uteroovarian veins in the rabbit and rat (Jones, 1977). Venoconstriction could cause progesterone to build up within the corpus luteum, activating its own feedback mechanism to stop progesterone production (McCracken, 1970). If this were true, vasodilators such as PGE_1 may have no particular effect on the corpus luteum.

In summary, vasodilators appear to have either no effect or luteotrophic effect when injected into PMSG/hCG primed rats. Vasoconstrictors, such as PGB_2 do not, however, appear necessarily luteolytic. Prostaglandin $F_2\alpha$, a potent vasoconstrictor, has been suspected of causing luteal demise by reducing blood supply to the corpus luteum. If this were true, vasoconstrictors such as PGB_2 would also cause luteolysis, but this did not happen in our experiment. We speculate that prostaglandin-induced luteolysis is different from luteolysis resulted from vasoconstriction but is the result of complex hormonal changes. Experiments using non-prostaglandin vasoconstrictors to study luteal function are underway in this laboratory.

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CURRENT DISTRIBUTION OF THE NATIVE BROOK TROUT IN THE APPALACHIAN REGION OF TENNESSEE

Rick D. Bivens, Richard J. Strange, and Douglas C. Peterson
The University of Tennessee
Knoxville, Tennessee 37901
and
Tennessee Wildlife Resources Agency
Talbott, Tennessee 37877

Abstract

Survey reports and field data of brook trout (*Salvelinus fontinalis*) inventories made from 1974 to 1984 were examined to determine the current distribution in Tennessee. Brook trout currently inhabit 275.6 kilometers in 135 streams of eight east Tennessee counties. Brook trout occur allopatrically in 195.7 kilometers and sympatrically with rainbow trout (*Salmo gairdneri*), and in some cases, brown trout (*Salmo trutta*), in another 79.9 kilometers. Thirty-two previously undocumented streams were found to have brook trout populations. Tennessee brook trout are generally found in small headwater streams above 925 meters elevation. These streams usually have soft water, low fertility, and are slightly acidic. Adult brook trout (>100 mm) collected in 1974-1984 from 41 streams had an overall mean total length of 151.5 mm, weight of 45.5 g, and condition factor (K) of 1.12.

Brook trout now occupy 20 to 30% of their estimated range in 1900. Habitat degradation from development projects, logging, forest fires, unregulated harvest, and introduction of exotic salmonid species have severely reduced the brook trout's range. Most of the loss probably occurred in the early 1900's, but recent surveys demonstrate that the process is ongoing. Current losses of brook trout populations are attributed mainly to the encroachment of rainbow trout as well as stream degradation. Only 33% of the current brook trout streams are known to have waterfall barriers that restrict the upstream movement of rainbow trout.

Introduction

The brook trout (*Salvelinus fontinalis*) is the only salmonid native to eastern North America and is near the southern limit of its natural range in Tennessee. Although its commonly accepted name is the brook trout, it is actually a member of the char genus, and often called the mountain or speckled trout. Brook trout populations have been declining in the southern Appalachians and especially in Tennessee since the early 1900's. Prehistorically, brook trout probably inhabited almost all streams on mountainous land throughout the Appalachian region of east Tennessee. Due to the influence of man, mainly through habitat degradation and the introduction of exotic trout species, the numbers and range of this southern trout have been severely reduced. King (1937) was among one of the first to note the change in distribution of this species in the Great Smoky Mountains National Park (GSMNP).

Due to its status as a prized game species and its importance to the native fish fauna of Tennessee, fisheries biologists and resource managers of various agencies began population inventories in the mid to late 1970's to determine the brook trout's current range and distribution. For