LYSOSOMAL ENZYME RELEASE FROM LUTEINIZED RAT OVARIES BY PROSTAGLANDIN $F_{2\alpha}$

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Extensive evidence has been amassed which demonstrates that prostaglandin (PG) $F_{2\alpha}$ plays an important role in the regulation of CL function. Recently, several experimental findings have buttressed the hypothesis that $PGF_{2\alpha}$ is the uterine luteolytic factor. It has been reported that $PGF_{2\alpha}$ induces degeneration of the CL of the pseudopregnant rat (Pharriss & Wyngarden, 1969), and other mammals (Duncan & Pharriss, 1970; Kirton *et al.*, 1970; McCracken *et al.*, 1970), that it decreases ovarian progesterone secretion in pregnant and pseudopregnant rats (Behrman *et al.*, 1971b), and that it is identifiable in uterine venous blood of guinea-pigs and sheep toward the end of the oestrous cycle, a time which coincides with luteal regression (Bland *et al.*, 1971; Blatchley *et al.*, 1972). Although these aforementioned data clearly demonstrate the luteolytic properties of $PGF_{2\alpha}$, the cellular mechanism(s) by which $PGF_{2\alpha}$ acts are poorly understood. In view of the findings that $PGF_{2\alpha}$ labilizes lysosomes of rat liver (Weiner & Kaley, 1972), pancreas, spleen and kidney cortex (Ignarro *et al.*, 1973), and that increased lysosomal enzyme activity occurs in rat lutein cells undergoing involution (Lobel *et al.*, 1961), the present study was undertaken to determine the effects of $PGF_{2\alpha}$ on acid hydrolase release from lysosomes of luteinized ovaries.

In order to obtain large amounts of relatively uniform luteal tissue, the superovulation procedure of Parlow (1958) was utilized. Immature female Holtzman rats 25 to 27 days old were treated with 50 i.u. PMSG followed 60 hr later with 25 i.u. HCG. The excessively luteinized ovaries were collected 7 days after ovulation, pooled, minced in vessels kept on cracked ice and homogenized in ice-cold 0.25 M-sucrose buffered to pH 7.4 with 0.01 M-tris using a motor-driven Teflon pestle and glass mortar. All subsequent preparative procedures were carried out at 0 to 4°C. A modification of the method of Dingle (1961) was employed to harvest lysosomes from the resultant homogenates. Ovarian homogenates were centrifuged at 800 g for 10 min to remove nuclei and unbroken cells. The supernatant was then centrifuged at 10,000 g for 20 min to sediment the large granule fraction containing lysosomes. This crude lysosomal pellet, which also contained microsomes, was resuspended in buffered sucrose and its protein concentration determined by the method of Lowry *et al.* (1951), employing bovine albumin as a standard. The suspension was then resedimented by centrifugation at 10,000 g for 20 min.

A pair of rat ovaries was taken to provide each 2.0 ml aliquot of lysosome-enriched suspension containing 1 mg granule protein/ml buffered sucrose. These lysosomal suspensions were incubated with PGF_{2α} or a solution (control) in which the PG was dissolved (trimethylol aminomethane, THAM, 6.8 µg/ml). Incubations were carried out for 30, 60, 90 and 120 min at 37°C with gentle shaking. After the incubation, suspensions were cooled on ice, centrifuged at 20,000 g for 20 min, and the activity of β-glucuronidase released into the supernatant was determined. Total β-glucuronidase activity was measured in the 20,000 g supernatant of an aliquot of lysosomal suspension which had been exposed to mechanical disruption in a Virtis homogenizer for 3 min and to thermal shock by five alternate freeze-thaw cycles.

The activity of β-glucuronidase (EC 3.2.1.31) was determined at pH 4.5 in acetate buffer utilizing phenolphthalein glucuronide as the substrate according to the procedure of Fishman *et al.* (1948). Released acid hydrolase, an index of lysosomal fragility, was calculated in units of µg phenolphthalein liberated/mg granule protein/hr at 37°C, and also expressed as the % of total β-glucuronidase activity.

Table 1. Effect of PGF_{2α} (20 µg/ml) on β-glucuronidase release from lysosomal suspensions of luteinized rat ovaries

No. of experiments	Time of incubation at 37°C (min)	% Total β-glucuronidase released	Difference from control	% Change from control*
15	30	20.07 ± 0.82†	+1.17 ± 0.23 (2P < 0.001‡)	+6.2 ± 1.2 (2P < 0.001)
15	60	23.09 ± 0.99†	+1.18 ± 0.49 (2P < 0.05)	+5.6 ± 2.1 (2P < 0.02)
15	90	26.39 ± 1.17†	+0.97 ± 0.33 (2P < 0.01)	+3.7 ± 1.2 (2P < 0.005)
15	120	32.50 ± 1.56†	+1.99 ± 0.40 (2P < 0.001)	+6.7 ± 1.2 (2P < 0.001)

All values are means ± S.E.M.

* Control release of β-glucuronidase assigned a value of 100.

† Release of β-glucuronidase significantly greater in each successive time period, 2P < 0.02, *t* test.

‡ *P* values were calculated by paired *t* test.

Supernatants of the lysosomal suspensions from the luteinized ovaries incubated for 30 min with buffered sucrose containing THAM (controls, *n* = 15) had 45.02 ± 3.21 (mean ± S.E.M.) β-glucuronidase units, which corresponded to 18.90 ± 0.77% of the total β-glucuronidase activity. The effects of PGF_{2α} on lysosomal fragility are depicted in Table 1. Lysosomal suspensions incubated with 20 µg PGF_{2α}/ml, showed a significantly increased release of lysosome-bound β-glucuronidase with time, and liberated significantly more enzyme than control suspensions at each incubation time studied. That the PGF_{2α}-induced increase in enzyme activity is due to a direct action on lysosomes is supported by the fact that PGF_{2α} had no demonstrable effect on β-glucuronidase activity when it was added to particle-free supernatants containing the enzyme (seven paired experiments).

Increased activity of lysosomal enzymes is an early event associated with luteal regression, and undoubtedly plays a pivotal role in the involution of the CL of the rat (Lobel *et al.*, 1961) and the ewe (Deane *et al.*, 1966). It has, moreover, been shown by Dingle *et al.* (1968) that lysosomes obtained from sheep CL undergoing regression exhibit a significantly greater fragility, as measured by the release *in vitro* of lysosomal marker enzymes in response to a standard physical stress, than those obtained from animals in early oestrus, or pregnancy. In the light of these experiments it was suggested that the uterine luteolytic factor affects lysosomes directly and that the resultant increase in enzyme leakage is perhaps the primary cause of regressive changes in the CL.

The ability of $\text{PGF}_{2\alpha}$ to initiate luteolysis when administered *in vivo* (Pharriss & Wyngarden, 1969) and its inability to do so when incubated with ovarian tissue *in vitro* (Sellner & Wickersham, 1970) led to the proposal of a possible vascular mechanism for $\text{PGF}_{2\alpha}$ action which was thought to be mediated by vasoconstriction of the uterine and utero-ovarian veins (Pharriss *et al.*, 1970). It would appear, however, that $\text{PGF}_{2\alpha}$ does not produce a sufficient reduction in blood flow to the CL to substantiate this mechanism (Bruce & Hillier, 1974). Furthermore, it has recently been demonstrated that $\text{PGF}_{2\alpha}$ markedly inhibits progesterone synthesis by rabbit CL *in vitro* (O'Grady *et al.*, 1972). These reports suggest a direct effect by $\text{PGF}_{2\alpha}$ on luteal tissue. The studies of Behrman *et al.* (1971a) imply that $\text{PGF}_{2\alpha}$ may produce a 'biochemical' lesion in the lutein cell which results in the lack of adequate storage of cholesterol esters and the consequent impaired ability of the cell to synthesize progesterone. The results of our studies, showing that $\text{PGF}_{2\alpha}$ labilizes lysosomes of luteinized rat ovaries, bridge the gap between the previously obtained biochemical and morphological findings and the putative physiological role of $\text{PGF}_{2\alpha}$ as the uterine luteolytic factor, and may provide an insight into the mechanism by which $\text{PGF}_{2\alpha}$ produces luteolysis *in vivo*.

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