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Lack of effect by prostaglandin $F_{2\alpha}$ and verapamil on calcium uptake by isolated corpora lutea from pseudopregnant rats

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Prostaglandin $F_{2\alpha}$ ($PGE_{2\alpha}$)* is of prime importance in initiating luteal regression in many species [1]. In isolated corpora lutea (CL) from pregnant and pseudopregnant rats [2–5], and in rat luteal cell suspensions [6, 7], $PGF_{2\alpha}$ rapidly abrogates the luteinizing hormone (LH)-induced cyclic AMP (cAMP) accumulation via an unclear mechanism. Behrman and his coworkers [7, 8] proposed that calcium ions mediate this action of $PGF_{2\alpha}$, based on the ability of ionophores and ouabain to inhibit cAMP accumulation in luteal cells. However, interference with extracellular [4, 5, 7] and intracellular [5] calcium did not prevent the $PGF_{2\alpha}$-induced suppression of cAMP accumulation. Furthermore, we found that calcium uptake is not affected by $PGF_{2\alpha}$ [4]; the latter study is extended here, using different schedules and CL ages.

Verapamil, a blocker of voltage-dependent calcium channels, has been applied to $PGF_{2\alpha}$-treated CL with the purpose of preventing extracellular calcium influx [4, 7]. However, verapamil-sensitive channels have not been demonstrated in rat CL. Therefore, the effect of verapamil on the uptake of $^{45}Ca^{2+}$ was also examined. For comparison, we tested the effect of lanthanum ions, which inhibit general calcium uptake as well as calcium loss from the cells [9, 10].

Materials and methods

$PGF_{2\alpha}$ (tromethamine salt) was a gift of the Upjohn Co., Kalamazoo, MI, U.S.A., and verapamil was donated by Knoll AG, Ludwigschafen am Rhein, Federal Republic of Germany. $^{45}CaCl_2$ (10–40 mCi/mg calcium) was a product of the Radiochemical Center, Amersham, England. The pregnant-mare-serum gonadotropin (PMSG) preparation was Gestyl (Organon, Oss, Holland). Lanthanum chloride was obtained from Sigma, and amino-acid and vitamin stock solutions were from Bio-Lab, Jerusalem, Israel.

The animal model and experimental procedures were described previously [4, 5]. Briefly, PMSG (15 I.U./rat, s.c.) was administered to 30-day-old female Sprague–Dawley rats; this resulted in the formation of CL which remained functional for 11 days. CL collected on day 10 or day 14 were pooled, and calcium uptake was determined according to published methods [4, 9, 10]. The incubation medium was very similar to Dulbecco's modified Eagle medium [4], and oxygen was bubbled constantly into the medium. CL were distributed in glass vials, or in test tubes, and incubated with $^{45}CaCl_2$ (2×10^5–10^6 cpm/ml) in the presence of various agents. When the effect of lanthanum ions on calcium uptake was examined, a modified medium was used, in which NaH_2PO_4, $NaHCO_3$ and $MgSO_4$ were omitted, and the final pH was 7.0. The calcium-depleted medium contained 0.5 mM ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), and $MgSO_4$ at 2.5 rather than 0.81 mM. The concentration of free calcium ions in such a medium is approximately 30 nM [5]. $LaCl_3$ and verapamil were dissolved in medium just before use. To terminate the incubation, CL were transferred into beakers with ice-cold wash solution containing 2 mM $LaCl_3$ [4], and, 50 min later, blotted, weighed in groups of 2–5 CL, and prepared for counting. Radioactivity in the incubation medium was also estimated. In CL held in the calcium-labeled medium for a few seconds, and then subjected to the described rinse procedure, the label was zero.

The methods for determining cAMP [5], 20α-hydroxysteroid dehydrogenase (20α-SDH) [11] and progesterone [12] have been described.

The specific activity of calcium ions taken up by the CL was assumed to be the same as that found in the medium (namely, 2×10^5–10^6 cpm/1.8 μmol). Calcium uptake was thus expressed as mmol/kg tissue (wet weight); sometimes, data from several experiments were combined. Values are presented as means ± SE, and statistical significance was determined by Student's *t*-test.

Results and discussion

The effect of $PGF_{2\alpha}$ on calcium uptake was examined in 14-day-old as well as 10-day-old CL. Day-10 CL were still functional, as indicated by the high concentration of plasma progesterone (284 ± 71 ng/ml, N = 7) and the negligible luteal 20α-SDH activity (0.31 ± 0.08 nmol/min/mg protein, N = 13). These mature CL are very sensitive to $PGF_{2\alpha}$ compared to young CL [3, 13]. On the other hand, 14-day-old CL had already undergone functional luteolysis: they produced very little progesterone (plasma level 9 ± 2 ng/ml, N = 9) and had high 20α-SDH activity (16.4 ± 3.7 nmol/min/mg protein, N = 9). A second generation of CL was not observed in any of the rats included in the study. Day-14 CL were examined because of the indications that $PGF_{2\alpha}$, beside triggering luteolysis in functional CL, also plays a role in later stages of luteal regression [1, 14, 15].

As shown in Table 1, $PGF_{2\alpha}$ added simultaneously with $^{45}CaCl_2$ had no effect on calcium uptake in CL of both ages; basal calcium uptake was similar in the two CL types. Responsiveness of the day-10 CL to $PGF_{2\alpha}$ (in terms of cAMP accumulation) was verified in groups of CL taken from the CL pool of each experiment. Mean cAMP values (pmol/mg protein) were 23.5 ± 5.1 in LH-treated CL, and 5.5 ± 0.8 in CL exposed to LH and $PGF_{2\alpha}$. In another experiment, the effect of $PGF_{2\alpha}$ on calcium uptake was

* Abbreviations: $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; LH, luteinizing hormone; cAMP, cyclic AMP; CL, corpora lutea; PMSG, pregnant-mare-serum gonadotropin; 20α-SDH, 20α-hydroxysteroid dehydrogenase; and EGTA, ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

Table 1. Effect of $PGF_{2\alpha}$ on calcium uptake in 10- and 14-day-old CL isolated from pseudopregnant rats

Age of CL	$PGF_{2\alpha}$ (μM)	Calcium uptake (mmol/kg tissue) 7 min	8 min	12 min	18 min
A. $^{45}Ca^{2+}$ and $PGF_{2\alpha}$ added at time zero*					
Day-10 CL	0	0.92 ± 0.05		1.33 ± 0.11	1.55 ± 0.10
	10	0.86 ± 0.05		1.30 ± 0.08	1.61 ± 0.11
Day-14 CL	0		1.09 ± 0.05	1.26 ± 0.10	1.68 ± 0.17
	10		1.03 ± 0.05	1.34 ± 0.08	1.63 ± 0.11
B. $^{45}Ca^{2+}$ added 150 min before $PGF_{2\alpha}$†					
Day-10 CL	0	11.73 ± 1.12		9.89 ± 0.54	14.16 ± 0.88
	10	10.67 ± 0.82		11.20 ± 1.25	13.27 ± 0.76

* $^{45}CaCl_2$—2 × 10^5 cpm/ml. Values are means ± SE. Data from day-10 CL were collected in four separate experiments (total N = 14–15 samples), and data from day-14 CL, in two separate experiments (total N = 10–12 samples).

† $^{45}CaCl_2$—6 × 10^5 cpm/ml. Values are means ± SE. N = 4 samples, 4–5 CL in each. Time of addition of $PGF_{2\alpha}$: t = 0.

examined in day-10 CL that had been preincubated for 150 min with radioactive calcium, and then incubated with and without $PGF_{2\alpha}$ for 7, 12 and 18 min. With this experimental design as well, $PGF_{2\alpha}$ had no effect on calcium uptake (Table 1). In a series of experiments not shown here, $PGF_{2\alpha}$ had no effect on calcium efflux in day-10 CL; $PGF_{2\alpha}$ was added after the CL had been preincubated with $^{45}CaCl_2$ (120 min), transferred through a series of test tubes with ice-cold rinse medium [10] to remove extracellular calcium (50 min), and returned to standard incubation conditions (10 min). However, after such pretreatment, luteal radioactive calcium content was 4.37 ± 0.27 mmol/kg tissue (N = 18), compared to a mean steady-state level of 11.82 mmol/kg tissue (Table 1). Thus, a calcium pool releasable by $PGF_{2\alpha}$ may have lost its label by then.

A lack of stimulation by $PGF_{2\alpha}$ of calcium fluxes would be compatible with our findings that in mature, functional CL from a slightly different model of pseudopregnant rats [3] $PGF_{2\alpha}$ does not stimulate polyphosphoinositide metabolism, although it does so in very young CL of the same model [16].

The exchangeable calcium pool, estimated by measuring $^{45}Ca^{2+}$ uptake in steady-state conditions (Table 1), is approximately 65% of total cell calcium, as measured by atomic absorption (171 nmol/mg protein, or approximately 19 mmol/kg tissue [5]).

To validate our experimental procedures, we measured calcium uptake in day-10 CL that had been preincubated for 90 min in either calcium-depleted (30 nM) or the standard medium (1.8 mM $CaCl_2$). We reported previously [5] that, after a 90-min incubation in the calcium-poor medium, total tissue calcium, measured by atomic absorption, decreased by 64%. In the present experiment, the preincubation was followed by a 2-min incubation in standard medium containing $^{45}Ca^{2+}$ (6 × 10^5 cpm/ml). For each treatment, four samples were prepared (4–5 CL/sample). Radioactive calcium uptake was much enhanced following calcium depletion, being 1.54 ± 0.23, compared to 0.46 ± 0.05 mmol/kg tissue in the controls ($P < 0.005$).

As another positive control, we measured calcium uptake in the presence of the calcium ionophore A23187 (5 μM), added simultaneously with $^{45}CaCl_2$ (10^6 cpm/ml, incubation time 2 min). Data from two experiments were combined after normalization of each value to the mean of the control values in the respective experiment (0.55 or 0.42 mmol/kg tissue). A modest but significant enhancement of calcium uptake by the ionophore was observed (128 ± 10, N = 15, vs 100 ± 5, N = 16, normalized units, $P < 0.025$). An effect of A23187 of similar magnitude was reported for pancreatic acini [17]; in that study, release by the ionophore of intracellularly-stored calcium was shown to reduce the measured net uptake of radioactivity.

As shown in Table 2, verapamil, a blocker of voltage-sensitive calcium channels, did not inhibit calcium uptake even when added 45 min prior to the isotope, at an effective concentration [18]. In contrast, calcium uptake was inhibited by lanthanum ions, which inhibit general calcium movement across the plasma membrane by replacing calcium on various binding sites [9, 10]. With 1 and 2 mM lanthanum, the inhibition became complete after less than 15 min. This effect of lanthanum is most probably a true inhibition of calcium uptake into the cell, since in the procedure used here all samples were ultimately exposed to ice-cold, lanthanum-containing wash solution, and thus extracellular calcium replaceable by lanthanum was removed from all samples to the same extent.

Table 2. Effects of verapamil and lanthanum ions on calcium uptake in 10-day-old CL isolated from pseudopregnant rats

	Inhibitor	Time of inhibitor addition	Calcium uptake (mmol/kg tissue) 15 min	30 min	45 min
Expt. 1	None		1.70 ± 0.06	2.63 ± 0.07	3.41 ± 0.15
	Verapamil (100 μM)	0	1.32 ± 0.11	2.67 ± 0.27	2.85 ± 0.36
	Verapamil (100 μM)	−45 min	1.51 ± 0.03	2.50 ± 0.24	4.07 ± 0.27
Expt. 2	None		1.35 ± 0.19	3.00 ± 0.36	
	$LaCl_3$ (1 mM)	0	0.74 ± 0.10*	0.86 ± 0.13†	
	$LaCl_3$ (2 mM)	0	0.52 ± 0.08‡	0.50 ± 0.10§	

Time of addition of $^{45}CaCl_2$ (10^6 cpm/ml): t = 0. Values are means ± SE; N = 4 samples, 3–6 CL in each.

*–§ Significantly different compared to the respective control: * $P < 0.05$, † $P < 0.005$, ‡ $P < 0.01$, and § $P < 0.001$ (Student's t-test).

These results suggest that rat CL may lack voltage-dependent calcium channels. Interestingly, in rat luteal cell suspension, the inhibitory effect of gonadotropin-releasing hormone on LH-induced cAMP accumulation is attenuated by depletion of extracellular calcium, but not by verapamil [7].

For the present study to be interpretable, one must assume that the whole luteal mass is accessible to components of the incubation medium. Diffusion of EGTA and calcium throughout the CL is very likely, since the fraction of cell calcium lost upon incubation in calcium-poor medium was identical in isolated CL [5] and in cultured granulosa cells [18]. Whole CL must be accessible also to $PGF_{2\alpha}$, since incubation of isolated CL with $PGF_{2\alpha}$ rendered adenylate cyclase activity in membranes from such CL virtually unresponsive to LH [19].

In summary, in CL from pseudopregnant rats, isolated either before or after functional luteolysis, $PGF_{2\alpha}$ did not stimulate calcium uptake. These results are in accord with other evidence, which suggests that extracellular and intracellular calcium does not mediate the suppression by $PGF_{2\alpha}$ of luteal cAMP. In functional CL, calcium uptake was not affected by verapamil, suggesting that voltage-dependent calcium channels are absent in rat CL. Validity of the experimental procedure was verified by the findings that calcium uptake was inhibited by lanthanum and stimulated by A23187 and by prior calcium depletion in the CL.

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Faculty of Medicine
The Technion-Israel
Institute of Technology
Haifa 31096, *Israel*

MICHAL LAHAV*
KOHAVA SHARIKI-SABAG
HANNA RENNERT

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* Corresponding author.