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INDUCTION OF DECIDUALIZATION IN PSEUDOPREGNANT HAMSTERS BY INTRAPERITONEAL INJECTION OF PROSTAGLANDIN F_{2a} AND HISTAMINE RELEASER (PYRATHIAZINE HYDROCHLORIDE)

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Summary

It is possible to induce decidualization in pseudopregnant hamsters without operation and manipulation of uterine tissues by intraperitioneal injection of 2–3 × 50 micrograms of PGF_{2a} or 20 mg of a histamine releaser (pyrathiazine hydrochloride) during 9.00 and 15.00 h of day 4 (D_4) of pseudopregnancy. Autopsy on D_9 showed maximal decidual induction score (DIS) and significant uterine and endometrium weight increments. Unlike PGF_{2a}, PGE_2 did not induce this response.

Indomethacin injection of 0.3–4.0 mg, either given as a single or multiple doses, was unable to effect complete inhibition of decidual cell reaction induced by uterine traumatization. Our evidence is in favor of the contention that both PGF_{2a} and histamine and neither alone may mediate the process of initiation and decidual formation in hamster.

Introduction

Systemic induction of decidualization in the laboratory rodents were introduced over 20 years ago[1, 2]. It was claimed that histamine was the only specific inducer for initiation of decidualization[3, 4], and induction of decidualization by systemic injection of histamine releasers should offer a new approach to follow up study on biochemical events in maternal tissues during the critical time for implantation[5]. A later report was unable to show consistent results of decidualization induced by systemic injection of pyrathiazine hydrochloride in the hamster[6]. Moreover, many workers have difficulties to correlate histamine theory with decidualization induced by intraluminal injection of non-specific agents including oil[7, 8] and air[9], although application of antihistamines showed complete inhibition of the decidualization in all cases[1, 10].

Prostaglandin (PG)F_{2a} was recently found to increase in the mouse endometrium during the critical time of transformation of uterine stromal cells into decidual cell[11–13], while histamine itself is synthesized by the rabbit blastocyst and plays significant role on stimulation of implantation in the rabbit[14, 15]. This study was undertaken to find

out whether or not the hamster, a typical mammalian model which does not require estrogen for induction of decidualization and nidation[16], could also repond to systemic injection of histamine releasers and/or prostaglandins and induce decidualization if injection is performed close to the time of maximal uterine sensitivity.

Materials and Methods

Two hundred and forty one female hamsters from the colony in the department were used. They were all virgin of 2–2½ months old and weighed 100–125 g Animals were kept in an air-conditioned room with daily light schedule of 10 hours darkness and 14 hours light. Tap water and rodent pallets (Gold Coin) were available *ad libitum*. Only animals having regular 4 days cycle for at least 2 cycles were used. Pseudopregnancy were induced by placing proestrous females overnight with vasectomized males and confirmed by the absence of the expected postestrous discharge of the next cycle. These animals were subjected to use for the following studies: 1) to determine if the hamster does have maximal uterine sensitivity period for response to trauma stimulation by scratching the entire length of uterine endometrium with a large needle at various intervals of D_4 and D_5, 2) study the effect of intraperitoneal injection of 10–20 mg pyrathiazine hydrochloride and/or PGs (PGF_{2a} & PGE_2) 50–150 μg or vehicle (0.4 ml) during the period of maximal uterine sensitivity observed in 1) and find out if any responses could be obtained, and 3) to see if indomethacin, a PG-synthetase inhibitor, prevents trauma-induced decidualization. PGs and pyrathiazine hydrochloride were dissolved in distilled water. Indomethacin was dissolved in olive oil. These drugs and vehicles were injected intraperitoneally at different intervals between D_2 and D_5. No toxic effect of these drugs were apparent in these experiments.

All animals were sacrified by cervical dislocation in the morning of D_9 of pseudopregnancy. Uterine horns and endometrium were removed, weighed and recorded for mean decidualization induction score (DIS) according to the method of Shelesnyak and Kraicer[2] as follows:

0 = no deciduomata;
1 = one-quarter or less of horn contains deciduomata;
2 = more than one-quarter but less than three-quarters of the horn contains deciduoma;
3 = three-quarters of the uterine length is decidual containing;
4 = entire uterine horn contains decidual tissue, most of the reaction in this grade fill the horn almost uniformly and give a 'sausage' appearance.

Results

It can be seen from Table 1 that maximal DIS response induced by traumatization could be obtained at any time of D_4. There was definite increment of uterine and endometrium weights of traumatized horn of all groups stimulated during 9.00 and 15.00 of D_4 but the response declined sharply in groups of animals stimulated on D_5.

TABLE 1. OPTIMAL TIME FOR INDUCTION OF DECIDUALIZATION BY TRAUMATIZATION THROUGH THE LEFT UTERINE HORN.

Traumatization time (hour)	D_9 Observation						
	Animals with decidualization/ Total (%)	Mean DIS[a]		Uterine weight (mg)		Endometrium weight (mg)	
		Traumatized horn	Control horn	Mean ± S.E.M. Traumatized horn	Control horn	Mean ± S.E.M. Treaumatized horn	Control horn
D_4 (9.00)	11/11 (100.0)	4.0	0.0	1167.6 ± 243[b]	362.1 ± 30.3	483.6 ± 98.8[b]	50.5 ± 15.8
(12.00)	12/12 (100.0)	4.0	0.0	1651.6 ± 128[b]	350.0 ± 21.0	795.9 ± 103,4[b]	59.2 ± 9.8
(15.00)	12/12 (100.0)	4.0	4.0	1701.2 ± 147[b]	386.1 ± 19.8	799.6 ± 95.7[b]	67.8 ± 11.1
D_5 (9.00)	7/9 (77.8)	3.0	0.0	725.8 ± 78[b]	436.6 ± 33 2	142.8 ± 44.7[b]	54.5 ± 12.1
(12.00)	3/9 (33.3)	0.8	0.0	473.2 ± 35[b]	392.8 ± 22.1	71.6 ± 20.4	45.8 ± 12.5
(15.00)	2/9 (22.2)	0.6	0.0	455.4 ± 53[b]	336.2 ± 39.2	43.9 ± 23.0	55.2 ± 23.0

[a] DIS = Decidualization Induction Score
[b] Significantly different from untraumatized (right) horn ($P < 0.01$).

TABLE 2. EFFECT OF INTRAPERITONEAL INJECTION OF PGF_{2a}, PGE_2 AND PYRATHIAZINE HYDROCHLORIDE ON INDUCTION OF DECIDUALIZATION

Treatment	D_9 Observation					
	Animals with decidualization/Total (%)	Mean DIS		Uterine weight (mg)	Endometrium weight (mg)	
		Left horn	Right horn	Mean ± S.E.M.	Mean ± S.E.M.	
1. Control						
D_4 (9.00 – 15.00)	0/10 (0.0)	0.0	0.0	444.0 ± 26.9	55.5 ± 2.6	
2. PGF_{2a}						
50 μg D_4 (12.00)	0/9 (0.0)	0.0	0.0	396.9 ± 4.6	42.6 ± 11.1	
50 × 2 μg D_4 (9.00 – 15.00)	7/8 (87.5)	3.5	3.5	533.3 ± 18.4[a]	157.6 ± 12.1	
50 × 3 μg D_4 (9.00 – 15.00)	8/8 (100.0)	4.0	4.0	585.4 ± 17.9[a]	167.5 ± 14.6[a]	
3. PGF_2						
50 μg D_4 (9.00)	0/10 (0.0)	0.0	0.0	516.8 ± 34.9	36.0 ± 3.4	
50 × 3 μg D_4 (9.00 – 15.00)	0/10 (0.0)	0.0	0.0	542.3 ± 55.4	66.0 ± 5.9	
4. Pyrathiazine injection						
15 mg D_4 (9.00)	0/7 (0.0)	0.0	0.0	593.8 ± 86.5	22.3 ± 3.4[b]	
15 mg D_4 (12.00)	0/7 (0.0)	0.0	0.0	522.9 ± 83.0	20.3 ± 3.6[b]	
20 mg D_4 (9.00)	6.9 (66.7)	2.6	2.6	683.5 ± 130.2[a]	15.4 ± 20.4[a]	
20 mg D_4 (15.00)	6/10 (60.0)	2.4	2.4	587.5 ± 74.3	96.7 ± 16.8[a]	
20 mg D_5 (9.00)	3/7 (42.9)	1.7	1.7	509.1 ± 84.6	56.6 ± 21.6	
20 mg D_5 (15.00)	0/9 (0.0)	0.0	0.0	504.3 ± 34.1	32.2 ± 5.5[b]	
5. PGF_{2a} & pyrathiazine injection at D_4 (12.00)						
PGF_{2a} 50 μg + Pyrathiazine 10 mg	5/9 (55.6)	2.2	2.2	470.2 ± 59.1	77.2 ± 12.1[a]	
PGF_{2a} 50 μg + Pyrathiazine 20 mg	4/9 (44.4)	1.8	1.8	298.1 ± 75.6	55.4 ± 16.6	

[a] Significantly higher than vehicle injected group ($P < 0.05$).
[b] Significantly les than vehicle injected group ($P < 0.05$).

Table 2 showed that intraperitoneal injection of PGF_{2a}, but not PGE_2, could stimulate decidualization only when injection was carried out more than one time. Maximal effective dose was 50×3 μg injected at 9.00, 12.00 and 15.00 hour of D_4 respectively. However, a single injection of 20 mg pyrathiazine on D_4 was sufficient to stimulate decidualization in the majority of animals observed. Surprisingly, animals which did not respond to pyrathiazine injection either at a smaller dose (15 mg) or at a time beyond the period of uterine sensitivity (D_5 15.00 h) showed significant reduction in endometrium weight from the vehicle injected group. It is of interest that simultaneous administration of 50 μg PGF_{2a} and pyrathiazine was sufficient to stimulate decidualization in many animals.

Table 3 showed that injections of 0.3–4.0 mg indomethacin at or a few days before the time of uterine traumatization were unable to show 100% inhibition of decidual cell reaction observed on D_9. Optimal doses for inhibiting the DIS and increment in uterine and endometrium weights were 1.2–2.0 mg, injected only in the morning and afternoon of D_4.

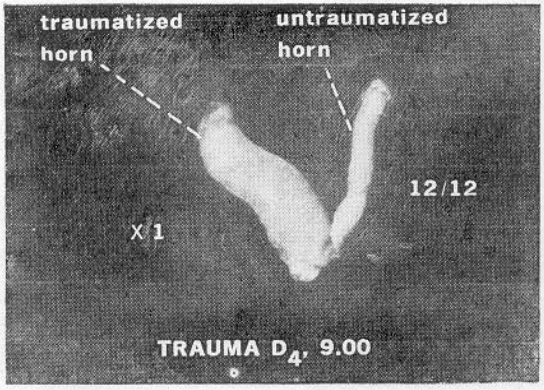
TABLE 3. EFFECT OF INTRAPERITONEAL INJECTION OF INDOMETHACIN ON INHIBITION OF D_4 TRAUMA INDUCTION OF DECIDUALIZATION

Treatment	D_9 Observation							
	Animals with decidualization/ Total (%)	Mean DIS		Uterine weight (mg)		Endometrium weight (mg)	
		Traumatized horn	Control horn	Mean ± S.E.M. Traumatized horn	Control horn	Mean ± S.E.M. Traumatized horn	Control horn
1. Uninjected control	35/35 (100.0)	4.0	0.0	1517.4 ± 170[a]	366.2 ± 13.8	699.6 ± 100[a]	60.0 ± 6.9
2. Indomethacin injection							
0.15 mg D_{2-5}	6/8 (75.0)	2.8	0.0	914.6 ± 275[a]	373.5 ± 50.8	309.1 ± 173[a]	95.2 ± 18.6
0.15 mg D_{4-5}	9/9 (100.0)	4.0	0.0	825.4 ± 95.5[a]	359.6 ± 20.7	244.0 ± 68.4[a]	72.2 ± 6.3
0.6 mg D_4 (9.00)	6/9 (66.7)	2.6	0.0	1569.6 ± 319[b]	446.4 ± 25.4	692.4 ± 203[a]	113.7 ± 13.2
0.6 mg×2 D_4 (9.00–15.00)	1/11 (9.1)	0.4	0.0	450.8 ± 42.5[b]	359.8 ± 36.9	40.6 ± 6.7	47.4 ± 10.0
1.0 mg×2 D_4 (9.00–15.00)	3/11 (27.3)	1.1	0.0	573.8 ± 27.1[a]	417.2 ± 11.2	87.9 ± 14.1	70.2 ± 6.6
2.0 mg×2 D_4 (9.00–15.00)	4/9 (44.4)	1.1	0.0	437.2 ± 12.8[a]	367.1 ± 8.1	52.2 ± 7.6	59.6 ± 9.6

[a] Significantly different from untraumatized (control) horn ($P < 0.01$).
[b] Significantly different from untraumatized (control) horn ($P < 0.05$).

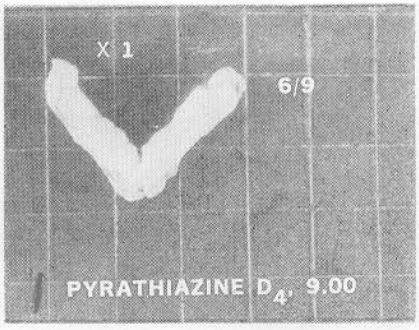
Discussion

This report is the first evidence to show that systemic induction of decidualization could successfully be demonstrated in a representative of mammalian species



A

Fig. 1. A A typical example of decidualized uterus (left) in D_9 animal previously traumatized through the uterine endometrium with a large and straight needle.



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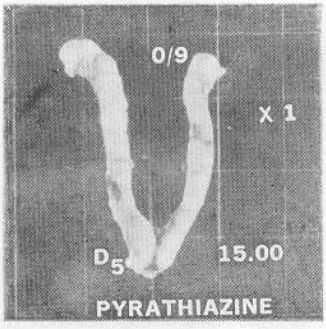
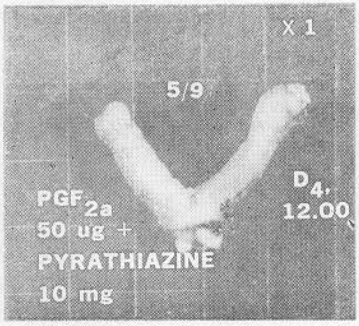


C

B. D_9 decidualized uteri of an animal with systemic injection of 20 mg pyrathiazine in the morning of D_4.

C. D_9 non-decidualized uteri of an animal treated with 20 mg pyrathiazine late in the afternoon of D_5.



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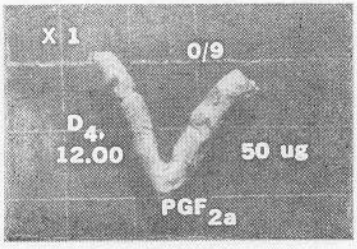


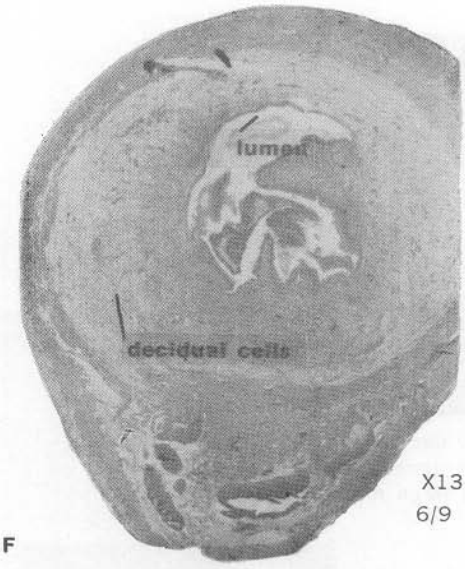
E

D. D_9 decidualized uteri of an animal with systemic injection of 50 μg PGF_{2a} and 10 mg pyrathiazine at noon of D_4.

E. Non-decidualized uteri of an animal treated with a single injection of 50 μg of PGF_{2a} at noon of D_4.

F. Cross section of the uterus with decidualization in animal shown in B.

G. Cross section of the uterus with no decidual cell reaction in animal shown in C.



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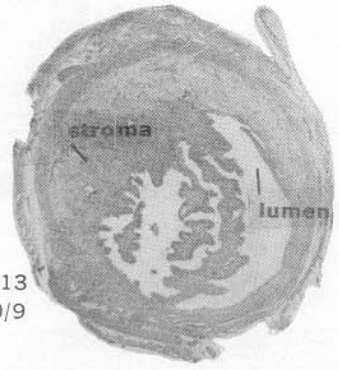

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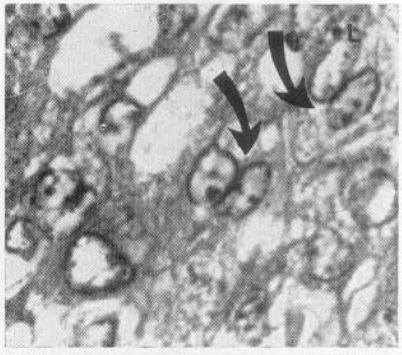

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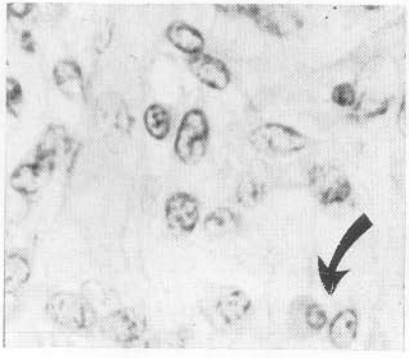
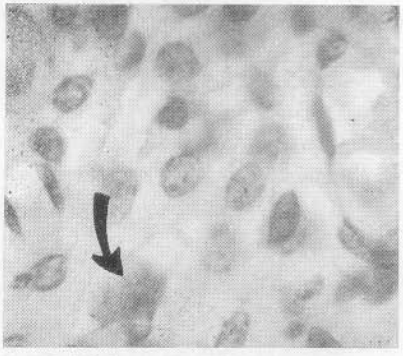


I



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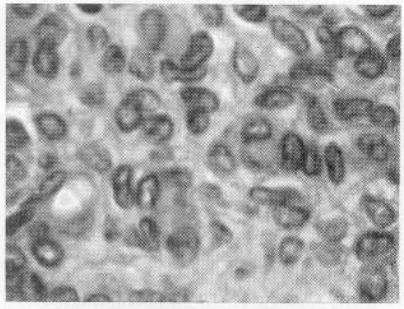

K

H.—J. Higher magnification of the cross section of decidualidualized uteri showing decidual cells in the animesometrial portion of animal shown in A(H) and B(I) and in an animal treated with 50 μg × 3 PGF_{2a} (J). Arrows in the figures show binucleated cells of the decidual tissue. There were no mitotic activities in all cases observed.

K. Higher magnification of the cross section of non-deciduated tissue from animals shown in G.

which do not require estrogen surge for stimulation of decidualization and nidation. Results were somewhat different from a previous report[6] which showed no decidualization in most hamsters injected intraperitoneally with pyrathiazine hydrochloride in the morning of D_5. This difference may probably be due to the fact that the maximal uterine sensitivity period to respond to initiation of decidualization in the hamster is approximately one day earlier than the rat and any stimulation given later than the evening of D_4 may be too late to obtain satisfactory response. Although the positive results of decidualization induction could be easily recognized at autopsy on D_9 by means of gross inspection of DIS as well as endometrium weight increment, the whole uterine wet weight of many treatment groups was not significantly increased from the control values of vehicle-injected group. This may probably be due to an extremely short life span of the hamster decidual tissue and 5 days post-stimulation may be beyond the peak period of growth (Orsini, M.W., personal communication). Lack of endomitotic activity of the decidual tissue observed in this study (Figure 1) fully supports this possibility.

It is interesting to note that both histamine releaser and PGF_{2a} (but not PGE_2) are able to stimulate decidualization in the hamster. Pyrathiazine requires much higher dose than PGF_{2a} but the latter compound needed to be applied at least 2–3 times to show positive response. This could be due to an extremely short half-life of PGs in circulating fluid[17] while a massive dose of pyrathiazine would be sufficient for stimulating the release of tissue histamine during the first 24 hours of injection[18].

The incapability of indomethacin in inhibiting decidual cell reaction induced by uterine trauma in many animals observed in this study and incomplete inhibition of nidation in rats previously depleted of tissue histamine[4] may suggest that neither PGF_{2a} nor histamine alone is the only factor responsible for stimulation of normal decidualization and blastocyst implantation.

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