PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 146, 912–917 (1974)

Effect of Prostaglandins E_1 and $F_{2\alpha}$ on the Isolated Mammary Gland of Nursing Rats (38218)

S. K. BATTA,[1] P. G. GAGLIANO, AND L. MARTINI

Department of Endocrinology, University of Milano, 32, Via Vanvitelli—20129 Milano, Italy

Very little attention has been paid so far to the possible effects of prostaglandins (PGs) on the mammary gland. PGE_1 has been shown to reduce oxytocin-induced increase of intramammary pressure in rabbits and in rats, but not to have any direct effect in the absence of oxytocin *in vivo* (1, 2). However, an isolated preparation of rat mammary gland studied in an isolated tissue bath responds to PGE_1 with a contraction (2). PGE_1, PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ have been reported to exert milk-ejecting effects in lactating guinea pigs (3).

The present study was undertaken in order to investigate *in vitro* the response of the mammary gland of lactating animals to two PGs (PGE_1 and $PGF_{2\alpha}$), to oxytocin and to different combined treatments. In this study, the mammary glands were collected from lactating female rats, dissected into fragments of the dimensions of approximately 1 mm^3, and placed on microslides. The fragments of the mammary glands were subsequently exposed to the effect of the compound(s) under investigation and the ejection of milk was observed under a magnifying lens. Two parameters were taken into consideration, namely (a) the percent modifications of the milk ejection responses observed; and (b) the interval between exposure to the different compounds and appearance of the response.

Materials and Methods. The method used was based on the *in vitro* bioassay of oxytocin described by Van Dongen and Hays (4). Lactating rats beyond the fifth day of lactation were used. The young were removed from the mother at least 20 hr before the initiation of the experiment. The mother was anesthetized with nembutal (4 mg/100 g body wt), and a part of one of the mammary glands excised and cleaned of all surrounding tissues. The excised mammary glands were placed immediately in De Jalon's solution at room temperature (NaCl, 9.0 g; KCl, 0.24 g; Na_2HCO_3 0.5 g; glucose, 1.0 g; and $CaCl_2$, 0.06 g dissolved in 1 liter distilled H_2O). They were subsequently dissected into small fragments (approximately 1 mm^3) and placed on microslides. The ejection of milk was observed under a magnifying lens. As previously stated 2 parameters were taken into consideration, i.e., the percent of fragments responding with milk ejection, and the time interval between exposure to each compound and appearance of the response. It is generally accepted that the milk is expelled out of the mammary gland by contraction of the myoepithelium, so that one may infer that the responses observed after the administration of the different compounds are due to an effect on the myoepithelium.

The following sets of experiments were performed:

Experiment N.1—The pieces of the mammary glands were placed on the microslides and various concentrations of oxytocin (1, 10, 100 mU/ml) added.

Experiment N.2—The breast tissue was exposed to PGE_1 (100 μg/ml) or to $PGF_{2\alpha}$ (100 μg/ml).

[1] Present address: Department of Obstetrics and Gynecology, University of Pennsylvania, School of Medicine—Philadelphia, Pennsylvania 19104—U.S.A.

Printed in U.S.A.

TABLE I. Effect of Various Concentrations of Oxytocin, PGE_1 and $PGF_{2\alpha}$ on the *in Vitro* Milk Ejection Response of the Mammary Gland.

	Groups[a]	% Incidence of responses	Time interval (seconds)
	OXYTOCIN		
A	1 mU/ml (79)	97.4	24.7 ± 1.0[b]
B	10 mU/ml (77)	96.1	15.0 ± 0.8
C	100 mU/ml (60)	98.3	9.3 ± 0.6
	PROSTAGLANDINS		
D	E_1 100 μg/ml (90)	33.3[c]	36.9 ± 2.7
E	$F_{2\alpha}$ 100 μg/ml (69)	56.5[d,e]	31.0 ± 2.3

[a] Number of observations in parentheses.
[b] Values are means ± S.E.
[c] D vs B: $P \leq 0.001$.
[d] E vs B: $P \leq 0.05$.
[e] D vs E: $P \leq 0.05$.

Experiment N.3—The isolated tissue was exposed to various concentrations (1, 10, 100 mU/ml) of oxytocin on a microslide. After a period of 90 sec, the tissue was removed from the medium containing oxytocin, and placed on another part of the slide and exposed to either PGE_1 (100 μg/ml) or $PGF_{2\alpha}$ (100 μg/ml).

Experiment N.4—In this set of experiments, the tissue was exposed to either PGE_1 (100 μg/ml) or $PGF_{2\alpha}$ (100 μg/ml) for 90 sec. The tissue was then removed, and exposed to different concentrations of oxytocin (1, 10, 100 mU/ml).

Experiment N.5—The tissue was exposed first to either 10 or 100 mU of oxytocin. After 90 sec, it was exposed again in a different part of the slide to the same doses of oxytocin.

Oxytocin was diluted in De Jalon's solution. PGE_1 was prepared by diluting a known amount in a drop of 95% ethanol; this solution was subsequently made up to a concentration of 100 μg/ml with normal saline. The tubes were sealed under nitrogen and stored frozen until used. $PGF_{2\alpha}$ was dissolved and diluted in normal saline to a concentration of 100 μg/ml, sealed under nitrogen and stored frozen until used.

Statistical analysis was carried out using X^2 test for the incidence of tissue responses and Student's *t* test for milk ejection time.

Results. Experiments N.1 and 2—In agreement with the results of Van Dongen and Hays (4), Table II shows that more than 95% of mammary gland fragments tested contracted under the influence of oxytocin irrespective of the dose used. There was a visible dose-response relationship on the other parameter considered (time interval between exposure to the hormone and appearance of the response). The effect of both PGs was much less pronounced than that of oxytocin both in terms of number of preparations responding and in terms of interval between exposure to the compound and appearance of the response. $PGF_{2\alpha}$ seemed to be more effective than PGE_1 in inducing an increased number of mammary gland fragments to respond. However, the interval between the exposure and the response was similar for both PGs.

Experiment N.3—The response of the mammary tissue to PGs is altered after the gland has been pretreated with oxytocin, as demonstrated in Table II. Pretreatment with oxytocin makes the preparation more sensitive to the effects of PGE_1. The number of fragments responding is significantly increased, while the delay of the milk ejection response is diminished. On one parameter, a correlation seems to exist between the dose of oxytocin used as a pretreatment and the potentiation of the response to PGE_1. It is apparent from Table II that after pretreatment with the highest dose of oxy-

TABLE II. Effect of Pretreatment with Oxytocin on the *in Vitro* Milk Ejection Response of the Mammary Gland to PGE_1 and $PGF_{2\alpha}$.

	Groups[a]	% Incidence of responses	Time interval (seconds)		Groups[a]	% Incidence of responses	Time interval (seconds)
A	Control (PGE_1 100 μg/ml alone)	(90)	33.3	36.9 ± 2.7[b]	A Control ($PGF_{2\alpha}$ 100 μg/ml alone)	(69)	56.5	31.0 ± 2.3[b]
	Oxytocin + PGE_1 (100 μg/ml)				Oxytocin + $PGF_{2\alpha}$ (100 μg/ml)			
B	1 mU/ml + "	(30)	67.7[c]	23.5 ± 2.5[c]	B 1 mU/ml + "	(18)	50.0	30.8 ± 7.2
C	10 mU/ml + "	(30)	77.4[d]	21.9 ± 3.3[c]	C 10 mU/ml + "	(19)	78.9	29.7 ± 5.8
D	100 mU/ml + "	(30)	53.5	15.1 ± 2.2[c]	D 100 mU/ml + "	(14)	71.4	27.4 ± 4.2

[a] Number of observations in parentheses.
[b] Values are means ± S.E.
[c] B vs A: $P \leq 0.05$.
[d] C vs A: $P \leq 0.01$.
[e] A vs B, C, D: $P \leq 0.005$.

tocin the response to PGE_1 occurs more rapidly. The effect of $PGF_{2\alpha}$ is not significantly influenced by pretreatment with oxytocin, even if the incidence of the milk ejection responses seems to increase after the tissue has been previously exposed to oxytocin.

Experiment N.4—The results obtained in this series of experiments are presented in Table III. The responsiveness to oxytocin of the fragments of mammary glands pretreated with either PGE_1 or $PGF_{2\alpha}$ is significantly reduced, when compared to that of fragments exposed to oxytocin in the absence of any pretreatment. After pretreatment with PGE_1 the number of fragments responding to oxytocin is decreased even if not significantly. On the other side, the time interval between exposure to oxytocin and the milk ejection response is significantly increased after pretreatment with PGE_1 irrespective of the concentrations of oxytocin used. After pretreatment with $PGF_{2\alpha}$, the interval between exposure to oxytocin and appearance of the response is enhanced, while the incidence of tissue responses is slightly diminished.

Experiment N.5—Pretreatment with oxytocin has no effect on a second response to oxytocin when the % of fragments responding is considered. However, following pretreatment with oxytocin a second exposure to the hormone results in a delayed response of the tissue. In other words, it seems that a pretreatment with oxytocin decreases the sensitivity of the preparation to a further administration of the compound.

Discussion. The data here presented demonstrate that PGE_1 and $PGF_{2\alpha}$ are able to induce *in vitro* the contraction of isolated mammary gland tissue taken from lactating rats and to provoke the ejection of milk. Furthermore, the data show that pretreatment with either PG reduces the sensitivity of the tissue to the effect of oxytocin. On the other hand, pretreatment with oxytocin increases the sensitivity of the mammary gland to PGs and especially to PGE_1. The potentiating effect oxytocin exerts on the contractions subsequently induced by PGs seems to be specific, since a second exposure to oxytocin does not result in a potentiation

TABLE III. Effect of Pretreatment with PGE_1 and $PGF_{2\alpha}$ on the *in Vitro* Milk Ejection Response of the Mammary Gland to Various Concentrations of Oxytocin.

Groups[a]	% Incidence of responses	Time interval (seconds)	Groups[a]	% Incidence of responses	Time interval (seconds)
Control (oxytocin alone)			Control (oxytocin alone)		
A 1 mU/ml (32)	96.8	24.4 ± 0.4[b]	A 1 mU/ml (24)	100.0	24.5 ± 1.9[b]
B 10 mU/ml (31)	96.7	13.5 ± 1.0	B 10 mU/ml (19)	100.0	16.7 ± 0.4
C 100 mU/ml (28)	100.0	8.8 ± 1.3	C 100 mU/ml (20)	95.0	8.3 ± 0.7
PGE_1 (100 μg/ml) + oxytocin			$PGF_{2\alpha}$ (100 μg/ml) + oxytocin		
D + 1 mU/ml (31)	70.0	38.1 ± 4.4[c]	D + 1 mU/ml (24)	90.4	40.5 ± 1.5[f]
E + 10 mU/ml (31)	80.5	31.2 ± 4.8[d]	E + 10 mU/ml (19)	76.4	25.1 ± 2.1[g]
F + 100 mU/ml (28)	79.5	38.1 ± 12.4[e]	F + 100 mU/ml (20)	87.5	17.8 ± 4.4[h]

[a] Number of observations in parentheses.
[b] Values are means ± S.E.
[c] D vs A: $P \leqslant 0.0025$.
[d] E vs B: $P \leqslant 0.0005$.
[e] F vs C: $P \leqslant 0.0125$.
[f] D vs A: $P \leqslant 0.0005$.
[g] E vs B: $P \leqslant 0.0005$.
[h] F vs C: $P \leqslant 0.0025$.

TABLE IV. Effect of Pretreatment with Oxytocin on the *in Vitro* Milk Ejection Response of the Mammary Gland to a Second Dose of Oxytocin.

Groups[a]		% Incidence of responses	Time interval (seconds)
Control (oxytocin)			
A	10 mU/ml (5)	100	14.6 ± 1.1[b]
B	100 mU/ml (6)	100	6.6 ± 0.3
Oxytocin + oxytocin			
C	+ 10 mU/ml (5)	100	22.4 ± 2.1[c]
D	+ 100 mU/ml (6)	83	24.4 ± 1.6[d]

[a] Number of observations in parentheses.
[b] Values are means ± S.E.
[c] C vs A: $P \leq 0.01$.
[d] D vs B: $P \leqslant 0.0005$.

of the oxytocin effect, but rather in a decreased responsiveness to the hormone. The observation that oxytocin pretreatment results in a better response of the mammary gland subsequently exposed to PGs, clearly indicates that the inhibition of the response to oxytocin observed following pretreatment with PGs is not due to exhaustion of the tissue due to a previous stimulation.

These data confirm a preceding study *in vitro* in which however a completely different technique was used (2) and agree with the *in vivo* observations of McNeilly and Fox (3) in lactating guinea pigs. The results here reported differ in several respects from those of Turker and Kiran (1) and of Haldar, Maiweg and Grosvenor (2). These authors were unable to demonstrate any direct effect of PGE_1 on the mammary gland of the rabbit and of the rat *in vivo*. Moreover, they reported that PGE_1 could antagonize the increase of intramammary pressure brought about by continuous infusions of oxytocin.

The data here presented seem to indicate that the relationships oxytocin PGs are more complex than it would appear from these *in vivo* observations. It has been shown indeed that the *in vitro* responses of the mammary gland to subsequent administrations of PGs and of oxytocin are not undirectional, and depend on the sequence of administration of the different compounds.

The data here presented do not permit to derive firm conclusions on whether oxytocin and PGs act on the same structures (myoepithelium?) and on the same cellular receptors. The possibility of different points of attack cannot be disregarded, mainly because of the opposite responses which are obtained when the sequences of administration of PGs and of oxytocin are reversed. This interpretation would be in line with the recent observations of Soloff and co-workers (5) which indicate that, at uterine level, oxytocin and PGs operate on different types of receptors.

Summary. PGE_1 and $PGF_{2\alpha}$ are able to induce *in vitro* the contraction of isolated mammary gland tissue taken from lactating rats, and to provoke the ejection of milk. *In vitro* pretreatment with either PG reduces the sensitivity of mammary gland tissue to oxytocin. *In vitro* pretreatment with oxytocin increases the sensitivity of the mammary gland to PGs and especially to PGE_1.

The work here described was supported by a Grant of the Ford Foundation, New York, N. Y.

The technical assistance of Mr. M. Vigliani is gratefully acknowledged.

Thanks are also due to the Upjohn Company, Kalamazoo, Michigan, for supplying PGE_1 and $PGF_{2\alpha}$.

1. Turker, R. K., and Kiran, B. K., Eur. J. Pharmacol. 8, 377 (1969).

2. Haldar, J., Maiweg, H., and Grosvenor, C. E., *in* "Program of the 52nd Meeting of the Endocrine Society," p. 130 (1970).

3. McNeilly, A. S., and Fox, C. A., J. Endocrinol. **51**, 603 (1971).

4. Van Dongen, C. G., and Hays, R. L., Endocrinology **78**, 1 (1966).

5. Soloff, M., Swartz, T., Morrison, M., and Saffran, M., Endocrinology **92**, 104 (1973).

Received Oct. 4, 1974. P.S.E.B.M., 1974, Vol. 146.