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Original article

The possible involvement of protein kinase $C_{(s)}$ and inositol phosphate metabolism in the basal but not in the prolactin stimulated casein release by the lactating rabbit mammary epithelial cell

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Summary — The secretagogue effect of prolactin (PRL) on casein release by epithelial mammary cells has been previously related to stimulation of the phospholipase A_2-arachidonic acid cascade. In order to determine whether other intracellular pathways are implicated in this secretagogue effect, different agents acting on protein kinase C (PKC) and phospholipase C (PLC) activity have been assessed *in vitro* in lactating rabbit mammary gland fragments. Phorbol ester (20 nM TPA) and 1-oleoyl-2 acetyl-*sn*-glycerol (10 μM (OAG) stimulated newly synthesized casein secretion and potentiated the PRL secretagogue effect. However, 100 μM quercetin, 100 μM H-7 and 5 and 20 nM staurosporine did not inhibit the latter effect. Exogenous PLC did not stimulate casein secretion. PRL did not affect production of inositol phosphates (IPs) during 10 or 60 min exposure. These results show that PKC activation may increase basal levels of casein secretion, and demonstrate that PRL does not act primarily *via* PKC activation or by PLC activation to stimulate casein secretion.

secretion / casein / prolactin / protein kinase C / phospholipase C

Résumé — La protéine kinase C et le métabolisme des phosphates d'inositol peuvent intervenir dans la sécrétion de base mais pas dans la sécrétion stimulée des caséines dans la cellule épithéliale mammaire de lapine en lactation. *L'effet stimulant de la prolactine (PRL) sur la sécrétion des caséines par la cellule épithéliale mammaire est étroitement relié à l'activation de la voie phospholipase A_2-acide arachidonique. Afin de vérifier si d'autres voies intracellulaires sont concernées dans la transmission de cet effet, différents agents stimulants ou inhibiteurs de la protéine kinase C (PKC) et de la phospholipase C (PLC) ont été ajoutés aux milieux d'incubation de fragments de glande mammaire de lapines en lactation. Un ester de phorbol (TPA, 20 nM) et le 1-oléoyl-2 acétyl–sn glycérol (OAG, 10 μM) stimulaient la sécrétion des caséines néosynthétisées et amplifiaient l'effet sécrétagogue de la PRL. Cependant, la quercetine (100 μM), H-7 (100 μM) et la staurosporine (5 et 20 nM) n'inhibaient pas cet effet. La PLC exogène ne stimulait pas la sécrétion des caséines. La PRL ne modifiait pas la production des phosphates d'inositol pendant les incubations de 10 à 60 min. Ces résultats montrent que la PKC peut augmenter le taux de base de la sécrétion des caséines et que la PRL n'agit pas, dans un premier temps, par une activation de la PKC ni par une activation de la PLC pour stimuler la sécrétion des caséines.*

sécrétion / caséine / prolactine / protéine kinase C / phospholipase C

INTRODUCTION

The anterior pituitary hormone, prolactin (PRL) has long been implicated in growth and differentiation of the mammary gland (Cowie, 1970). In addition, release of PRL by suckling in rabbits accounts for high milk yields during early lactation and for maintenance of lactation (Mena *et al,* 1974; Taylor and Peaker, 1975). Moreover, PRL exerts a direct effect (secretagogue effect) on the release of caseins by epithelial mammary cells (Ollivier-Bousquet, 1978).

PRL belongs to a protein family which also includes GH, erythropoietin, and interleukins. They have common biological properties; *ie* they bind to receptors which present high structural similarities (D'Andrea *et al,* 990). The transduction signal pathways by which these factor–receptor complexes elicit effects at the cellular level are still very poorly known. Several lines of evidence suggest that tyrosine phosphorylation is a component in the interleukin 2 signalling mechanism (Mills *et al,* 1990). However, phospholipases A_2 and C (PLA_2 and PLC) are implicated in the action of erythropoietin, the activation of PLA_2 resulting in a rise in lipoxygenase metabolites of arachidonic acid (AA), and the activation of PLC resulting in the activation of protein kinase C (PKC) (Mason-Garcia and Beckman, 1991).

Despite the fact that most of the classical intracellular signalling molecules have been studied, a number of aspects concerning the mechanisms of the different effects of PRL remain controversial (Rillema, 1980; Bolander, 1985; Devinoy *et al,* 1988). Under PRL action transient and delayed hydrolysis of phospholipids occurs within 60 min in mammary gland explants of pregnant mice. But this delayed effect does not seem compatible with the primary action of the hormone on differentiation (Etindi and Rillema, 1988). An intracellular action of PRL could be related to activation of PKC, as attested by the effects of tumor promotor (2-*O*-tetradecanoyl-phorbol-13-acetate) in mammary gland explants (Waters and Rillema, 1989), in isolated nuclei from rat liver (Buckley *et al,* 1987, 1988), and by the effect of an inhibitor of PKC activity, gossypol, on differentiation of mouse mammary cells induced by PRL (Etindi and Rillema, 1987).

Intracellular signalling of the secretagogue effect of PRL has been studied in lactating rabbit mammary gland. Cyclic AMP accumulation does not participate directly in signal transmission of PRL hormonal action in the mammary gland (Ollivier-Bousquet, 1989). However, it has been shown that PLA_2 activation might intervene in this effect. PRL induces an early rise in AA, and the integrity of the metabolic transformation of this fatty acid by lipoxygenases and cyclooxygenases is necessary for the secretagogue response (Ollivier-Bousquet, 1984; Blachier *et al,* 1988). Is this cascade of events one of the first effects after binding of PRL to its receptor; or is it the consequence of the generation of intracellular mediators such as diacylglycerol (an activator of PKC), and inositol phosphates (IPs) resulting from the activation of PLC?

In order to determine more precisely the possible role of intracellular signalling of PRL secretagogue action by PLC or protein kinase C, various agents acting on these molecules have been examined. Diacylglycerol (in the 1-oleoyl-2 acetyl-*sn*-glycerol (OAG) form, able to penetrate the cells) is an endogenous activator of PKC (Kikkawa and Nishiguka, 1986; and phorbol esters mimic this effect (Castagna *et al,* 1982). H-7 (Hidaka *et al,* 1984), quercetin (Caufield and Bolander, 1986), staurosporine (Tamaoki *et al,* 1986) have been reported to inhibit PKC activity. Neomycin

exerts an inhibitory action on phosphoinositide metabolism (Schacht, 1978). All these agents were added to the incubation medium of mammary gland fragments and their effects on basal or stimulated casein secretion measured. Moreover, PRL and carbamylcholine were compared as potential elicitors of intracellular IPs accumulation.

MATERIALS AND METHODS

Fragments from New Zealand rabbit doe mammary gland were used at the 15th d of lactation. Litters were standardized to 8 animals and the last suckling was allowed to take place 2 h prior to the experiment. Each experiment was performed with mammary tissue fragments from 1 animal.

Incubation

Tissue fragments (each weighing 0.1–0.2 mg; total weight per assay: 5–10 mg) were incubated in Hanks' medium (pH 7.4; 37 °C, atm 95% O_2, 5% CO_2) containing bicarbonate (2.2 g/l).

Casein labelling

After 30 min preincubation in Hanks' medium the tissue fragments were pulse-labelled for 3 min with 1 480 kBq/ml of L-(3, 4, 5-^{3}H) leucine (CEA Saclay, France, specific act 2.22 TBq/mmol), rinsed extensively with the medium and reincubated for 60 min after start of the pulse with or without 10 µg/ml ovine PRL (kindly provided by NIADDK, NIH, Bethesda, MD, USA), the agent tested, or a combination of PRL + the agent tested. The agents tested were 10 µM OAG, 20 nM 12-*O*-tetradecanoyl phorbol 13-acetate (TPA), 100 µM 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H-7), 100 µM quercetin, 5–200 nM staurosporine, 0.1 and 1 mM neomycin, phospholipase C from *Bacillus cereus* and phospholipase C from *Bacillus thuringensis*. All these agents were obtained from Sigma (St Louis, MO, USA).

Labelled casein assay

The incubations were stopped 60 min after initial labelling and the tissues weighed and homogenized in 300 µl 10 mM phosphate-buffered saline (PBS) (pH 7.2), 1% Triton and 0.5% sodium deoxycholate; tissue proteins were precipitated with 300 µl 20% trichloroacetic acid in the presence of 50 µl 1% bovine serum albumin and washed with 10% trichloroacetic acid. The labelled caseins secreted in the medium were precipitated at their isoelectric point with 0.5 M sodium acetate buffer (pH 4.6) in the presence of non-labelled caseins (final dilution 0.5 mg/ml). The radioactivity of caseins secreted per mg tissue and tissue proteins per mg tissue was assessed by liquid scintillation counting in a Packard spectrometer. The results were expressed as the percentage of radioactivity secreted *versus* the total radioactivity incorporated into the cells.

Secreted caseins (%)

= {radioactivity of secreted caseins (cpm/mg)

x [radioactivity of tissue protein (cpm/mg)

+ radioactivity of secreted casein (cpm/mg)]$^{-1}$

x 100.

Inositol phosphate labelling

After 30 min of preincubation in Hanks' medium, the tissue fragments were labelled for 2 h with 370 kBq/ml of myo-^{3}H-inositol (Amersham, Bucks UK) extensively washed with the same medium containing 1 mM unlabelled myo-inositol and 5 mM LiCl. Tissue fragments (30–50 mg) were incubated in 1 ml of the same medium for 10 min and 1 h, in the presence or not of prolactin (10 µg/ml) 100 µM carbamylcholine (Sigma, St Louis, MO), 5 µM epinephrine (Sigma, St Louis, MO) and 30 µM propranolol (Sigma, St Louis, MO).

Extraction of lipids and water-soluble compounds

At the end of the incubation period and after addition of 3 ml CH Cl_3/CH_3 OH HCl (12 N) to the tubes containing tissues plus medium (2/1/0.01,

v/v/v) and vigorous agitation, the tubes were centrifuged (1 min, 800 g).

Chromatography of inositol phosphate

An aliquot (0.6 ml) of the upper aqueous phase was mixed with 2 ml H_2O and neutralized with 0.17 ml of a solution of 0.3 M Tris and applied to a AGI– X 8 column (1 ml 200–400, formate form).

Glycerophosphoinositol and inositol mono-, bi-, tri- and tetrakisphosphate were eluted from the column in a stepwise manner as described by Berridge *et al* (1983). Briefly, ^{3}H-inositol, glycerophosphoinositol, and inositol mono-, bi-, tri- and tetrakisphosphate were sequentially eluted by 3 x 3 ml H_2O, 4 x 3 ml of 60 mM ammonium formate, 5 mM sodium tetraborate solution, 4 x 3 ml 0.1 N formic acid plus 0.2 N ammonium formate, 3 x 3 ml 0.1 N formic acid plus 0.4 M ammonium formate, 3 x 3 ml 0.1 N formic acid plus 0.8 M ammonium formate then 3 x 3 ml 0.1 N formic acid plus 1.2 M ammonium formate. Eluates were examined for their radioactive content by liquid scintillation.

Statistical analysis

Each experiment was performed with mammary gland fragments from 1 doe. Control and treated assays differed only by adding the agent or inhibitor tested. Student's *t*-test on paired differences was used to statistically evaluate the differences between the treated and the control groups.

RESULTS

Action of TPA, OAG and PKC inhibitors on casein secretion

Ten μM OAG and 20 nM TPA stimulated newly synthesized casein secretion to the same extent as PRL. Combined with PRL, OAG and TPA potentiated PRL increase (fig 1).

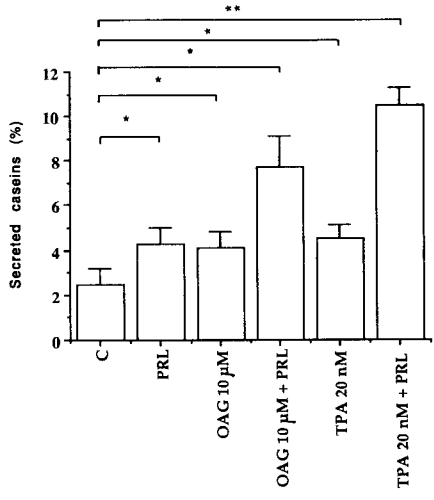

Fig 1. Effect of 10 μM OAG and 20 nM TPA on basal and PRL stimulated casein secretion. Mammary tissue fragments were labelled for 3 min with L- [^{3}H]-leucine, washed and incubated in the presence or not (control, C) of 10 μg/ml of PRL and the agents tested. Sixty min after the beginning of the pulse, radioactive secreted caseins were quantified as described in *Materials and Methods*. Mean ± SEM from 4 animals. * $P < 0.05$; ** $P < 0.02$.

Quercetin at a concentration of 100 μM is a specific inhibitor of PKC in mouse mammary gland explants (Caufield and Bolander, 1986). In the presence of 100 μM quercetin, the basal level of newly synthesized casein secretion was not affected and PRL exerted its secretagogue effect. Another inhibitor of PKC, 100 μM H-7 had no inhibitory effect on PRL secretagogue action: however, this agent slightly increased the basal level of secretion and markedly potentiated the secretory response to PRL (fig 2).

In order to determine the possible involvement of PKC in the basal release of casein, the effects of association of OAG and quercetin on secretion were measured. The casein secretion ratios were re-

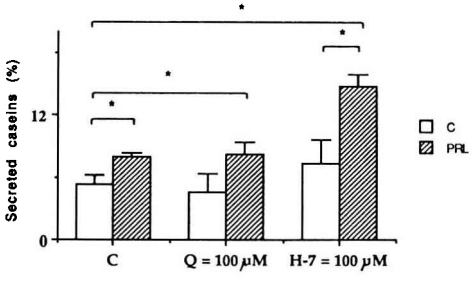

Fig 2. Effect of 100 μM quercetin (Q) and 100 μmol.l^{-1} H-7 on basal and PRL stimulated casein secretion. Mammary tissue fragments were labelled for 3 min with L-[^{3}H]-leucine, washed and incubated in the presence or not (control, C) of 10 μg/ml of PRL and the agent tested. Sixty min after the beginning of the pulse, radioactive secreted caseins were evaluated as described in *Materials and Methods.* Mean ± SEM from 4 animals; * $P < 0.05$.

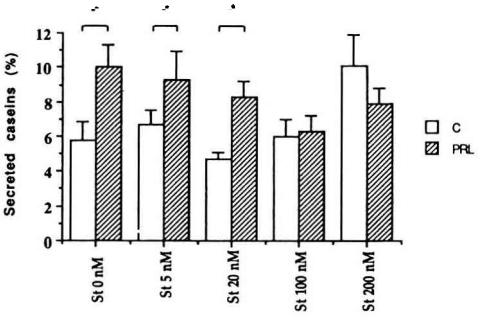

Fig 3. Effect of staurosporine on basal andPRL stimulated casein secretion. Mammary tissue fragments were labelled for 3 min with L-[^{3}H]-leucine, washed and incubated in the presence or not (control, C) of 10 μg/ml of PRL and various concentrations of staurosporine (St). Sixty min after the beginning of the pulse, radioactive secreted caseins were evaluated as described in *Materials and Methods.* Mean ± SEM from 4 animals; * $P < 0.05$.

spectively 4.5 ± 1 and 3.0 ± 0.4 (7 animals), in the presence of 10 μM OAG and 10 μM OAG + 100 μM quercetin. This result shows that quercetin is able to inhibit the stimulatory effect of OAG on the release of casein (compared to the basal level shown in fig 1). This suggests that PKC is implied in the stimulation induced by OAG.

Staurosporine is considered as a potent inhibitor of protein kinase C acting at a nanomolar concentration. Figure 3 shows that when added at a concentration of 5 nM and 20 nM to the incubation medium staurosporine did not significantly modify the basal level or the stimulated level of secretion. At a concentration of 100 nM and 200 nM, staurosporine impaired the PRL secretagogue effect. At a concentration of 200 nM, the drug stimulated the basal level of casein secretion. In consequence, the action of staurosporine at low concentrations described as selectively inhibitory of PKC were not observed. Inhibition was evident only at higher concentrations.

The above results are compatible with a possible action of PKC on the secretory mechanism itself, but suggest that inhibition of PKC activity does not directly interfere with the PRL secretagogue effect.

Action of PLC on casein secretion

In order to determine a possible role of PLC on casein secretion by hydrolysis of phosphatidylinositol in cellular membranes, PLC from *Bacillus cereus* and *Bacillus thuringensis* (not shown) were added to the incubation medium. Neither of these PLC exerted a stimulatory effect on newly synthesized secretion within the range of concentrations used (10–1 000 mUI) (fig 4). These results show that exogenous PLC was not able to mimic the PRL secretagogue effect.

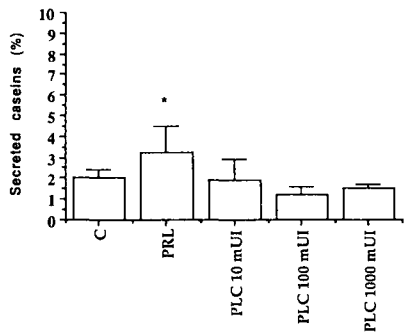

Fig 4. Effect of phospholipase C (PLC) from *Bacillus cereus* on casein secretion. Mammary tissue fragments were labelled for 3 min with L-[^{3}H]-leucine, washed and incubated in the presence or not (control, C) of 10 μg/ml of PRL and 10 –1 000 mUI of PLC. Sixty min after the beginning of the pulse, radioactive secreted caseins were evaluated as described in *Materials and Methods*. Mean ± SEM from 4 animals; * $P < 0.05$.

Because neomycin has been described as an inhibitor of phosphoinositide metabolism, this agent was added to the incubation medium of mammary gland fragments. Table I shows that in the presence of 0.1 mM or 1 mM neomycin, the basal level of newly synthesized casein secretion was not affected. After 30 min incubation, PRL exerted its secretagogue effect in the presence of 0.1 mM or 1 mM neomycin. After 60 min incubation, neomycin did not affect PRL effect at a concentration of 0.1 mM but was inhibitory at a concentration of 1 mM. Inhibition of phosphoinositide metabolism did not interfere markedly in the early effect of PRL. The only effect observed was a more delayed effect (60 min at high concentration) on the metabolism of the cell.

Since the lack of effect of PLC, when applied externally, does not constitute definitive proof that PLC is not involved in the response to PRL, the association of this experimental approach with the effect of neomycin suggests that the early effect of PRL does not imply phosphoinositide metabolism.

Action of PRL on intracellular accumulation of inositol phosphates

To evaluate a possible direct regulation by PRL of inositol metabolism, the content of ^{3}H-inositol phosphates was determined after labelling with ^{3}H-myo-inositol and incubating in the presence or not of PRL. Production of ^{3}H present in inositol monophosphate (IP_1), inositol biphosphate

Table I. Effect of neomycin on basal and PRL-stimulated casein secretion.

Incubation medium	*30 min (5)*	*60 min (10)*
Control	2.4 ± 0.4	6.6 ± 1.4
PRL (10 μg/ml)	3.7 ± 0.6*	9.4 ± 1.2*
Neomycin (0.1 mM)	2.9 ± 0.9	5.3 ± 1.3
Neomycin (0.1 mM) + PRL (10 μg/ml)	4.8 ± 0.6*	8.2 ± 1.3*
Neomycin 1 mM	2.4 ± 0.5	5.2 ± 1.1
Neomycin (1 mM) + PRL (10 μg/ml)	4.2 ± 0.2*	6.4 ± 1.0

Radioactive secreted caseins (%) were quantified as described in *Materials and Methods*. In brackets: number of animals; * $P < 0.05$.

(IP_2), inositol triphosphate (IP_3) inositol tetrakisphosphate (IP_4) were measured. Prolactin did not affect the amount of IP_1, IP_2, IP_3 and IP_4 production over a 10- or 60-min exposure period (fig 5). The question arises of whether phosphoinositide metabolism in lactating rabbit mammary gland is regulated by another hormonal control. It is well known that carbamylcholine stimulates the production of IPs in endocrine and exocrine pancreas (Blachier and Malaisse, 1990). One hundred μM carbamylcholine did not modify IPs production during a 10-min exposure period (data not shown). In contrast, in the same experimental conditions 100 μM carbamylcholine was able to increase the production of IPs by incubated pancreatic fragments during a 10 or 60 min exposure period. Five μM epinephrine was able to slightly increase the production of IPs. Five μM epinephrine plus 30 μM propranolol (a β-adrenoreceptor blocker) did not modify production of IPs during a 10-min exposure period (fig 5).

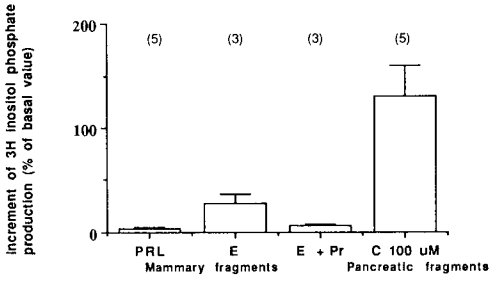

Fig 5. Effect of 10 μg/ml PRL, 5 μM epinephrine (E) and 5 μM epinephrine + 30 μM propranolol (E + Pr) on [^{3}H]-myo-inositol phosphate production by mammary tissue fragments and effect of 100 μM carbamylcholine (C) on [^{3}H]-inositol phosphate production by pancreatic fragments. Tissue fragments were incubated for 2 h in the presence of [^{3}H]-myo-inositol, washed and incubated in the presence of the different agents tested. [^{3}H]-Inositol phosphate production was measured after 10 min of incubation; in brackets: number of animals.

DISCUSSION AND CONCLUSION

Transmission of the hormonal signal in a cell can be effected by different mechanisms. PRL secretagogue effect in the mammary epithelial cell is largely dependent on an intracellular pathway implicating PLA_2 activation, release and metabolism of AA (Blachier *et al,* 1988). However, other intracellular mechanisms of signal transmission such as activation of PLC and of protein kinase C could intervene as a primary effect of PRL after fixation to its receptor and generate intracellular messengers able to secondarily activate PLA_2. Present results clearly show that neither PKC nor PLC seem to be involved in a primary effect of PRL, but they could be implicated in the regulation of basal secretion in the mammary cell.

Protein kinase C plays a role in many cellular processes involved in hormone-induced cell activation; an diacylglycerol, produced in many cases of IP receptor-mediated cleavage is an endogenous activator of protein kinase C (Kikkawa and Nishizuka, 1986). Phorbol esters mimic the stimulatory effect of diacylglycerol on protein kinase C (Castagna *et al,* 1982). These agents are able to stimulate secretion (Merrit and Rubin, 1985). In lactating rabbit mammary epithelial cells, OAG and TPA are also able to increase newly synthesized casein secretion and also potentiate the PRL effect. These result suggest that protein kinase C could be acting on basal casein secretion. Many compounds such as quercetin, H-7 and staurosporine have been reported to inhibit protein kinase C. One hundred μM quercetin inhibits protein kinase C activity in mice mammary gland explants (Caufield and Bolander, 1986). At this concentration, this inhibitor did not modify the basal level of casein secretion or the stimulating effect of PRL in lactating rabbit mammary

epithelial cells but inhibited OAG-stimulated secretion. H-7 has been used to inhibit the effect of phorbol esters in several tissues (Hidaka and Hagiwara, 1987). At a concentration of 100 μM, it exerted a slight stimulatory effect on the release of newly-synthesized caseins in mammary cells and potentiated the PRL secretagogue effect. It should be noted that this compound, which inhibits acinar protein kinase C activity in rabbit pancreatic acini, also potentiates stimulated pancreatic amylase secretion (Pandol and Schoeffield, 1986; Ederveen *et al,* 1990). The latter authors concluded that protein kinase C could exert a negative feedback role in receptor-mediated pancreatic enzyme secretion. However, as regards pancreatic secretion (Ederveen *et al,* 1990) and mammary epithelial cell secretion, this hypothesis is not supported by the results obtained with other putative protein kinase C inhibitors.

Staurosporine is inhibitory at nmol concentrations, acting on the catalytic domain of protein kinase C (Tamaoki *et al,* 1986). It has been shown to dose-dependently inhibit the pancreatic secretory response (Ederveen *et al,* 1990). This agent when added at nmol concentrations to the incubation medium of lactating mammary epithelial cells did not modify basal and PRL-stimulated casein secretion. At a concentration of 100 nM it inhibited the PRL effect, at 200 nM it increased the basal secretion level and PRL was no longer able to increase secretion. Interpretation of these results remains problematic because of the poor specificity of staurosporine which has been shown to inhibit kinases other than protein kinase C (Boyer *et al,* 1991). The latter authors emphasize an inhibitory effect on kinases able to control internalization processes. PRL internalization could be important for the transmission of its secretagogue effect (Seddiki and Ollivier-Bousquet, 1991). More investigation is therefore required on the effects of this inhibitor in the mammary cell. It appears that phorbol ester or OAG-sensitive protein kinases are not strictly involved in PRL-induced increased secretion. Interestingly, in the same manner, in anterior pituitary cells, a phorbol ester-sensitive protein kinase may increase PRL-secretion; but this enzyme may not be directly involved in the stimulatory effect on TRH on PRL secretion (Judd *et al,* 1989). Lastly, the metabolic role of protein kinase C in the mammary epithelial cells needs further investigation. It remains to be seen whether this enzyme has a different role during pregnancy when protein kinase C activity is high than during lactation when this activity is very low (Caufield and Bolander, 1986).

After activation through hormone PLC, a ubiquitous membrane-associated transducing enzyme, leads to the breakdown of phosphatidylinositol phosphates, generating second messengers such as IPs and diacylglycerol (Berridge, 1984; Berridge and Irvine, 1984). A manner in which prolactin stimulates lactogenesis could be through receptor-linked phosphoinositide hydrolysis (Rillema, 1985; Rillema and Waters, 1986). However, in cultured pregnant mouse mammary gland explants, a stimulatory effect of PRL on the accumulation of ^{3}H label in IPs does not appear before 1 h; this excludes a primary action of prolactin on lactogenesis that affects phosphoinositide metabolism (Etindi and Rillema, 1988). Since PRL has different actions on mammary epithelial cells, depending on the physiological stage, it was of interest to determine whether the secretagogue effect of PRL on lactating rabbit mammary epithelial cells was mediated by activation of PLC and increased IPs production. Present results clearly show that exogenous PLC does not mimic this PRL effect. Neomy-

cin, known for its inhibitory action on phosphoinositide metabolism does not inhibit PRL effect. Finally, PRL does not increase IPs production by lactating rabbit mammary epithelial cells over a 10- or 60-min period. An early effect of PRL was previously detected in another pathway, the AA cascade, as early as 1 min after addition of the hormone. Since no effect is detectable on the products of the PLC-activated pathway, it seems that a primary effect of PRL does not implicate phosphoinositide metabolism. It cannot be excluded that a minor change might be masked by the basal level of IPs. However, in the same experimental condition carbamylcholine stimulation of IPs production in pancreatic tissues is clearly detectable as early as 10 min and IPs accumulate, in agreement with the results of Blachier and Malaisse (1990). Epinephrine is able to slightly increase IPs production, likely by acting *via* the β-adrenergic receptors. These receptors are present in mammary epithelial cells (Clegg, 1987) and after binding to these receptors the main effect of epinephrine is to activate the cAMP pathway. The present results suggest that interactions between different pathways in the cell must be considered.

In conclusion, we have shown that in the lactating rabbit mammary gland, agents activating protein kinase C positively modulate the secretion of newly synthesized caseins. However, this enzyme does not appear to be a direct mediator of the secretagogue effect of PRL. Moreover, PRL does not increase IPs production and PLC does not mimic the secretagogue effect of PRL. It is hypothesized that these enzymatic pathways could be involved in basal metabolic processes of secretion. Questions remain on the possible intracellular interactions between these enzymatic pathways and the intracellular signalling of the PRL secretagogue effect by the AA cascade.

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