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Original article

The possible involvement of tubulin in transduction of the prolactin signal

LM Houdebine

INRA, Unité de Différenciation Cellulaire, 78350 Jouy-en-Josas, France

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Summary — Polactin has many different biological functions. It stimulates Nb_2 cell multiplication through the kinase C transduction mechanism, casein secretion through the phospholipase A_2-arachidonic acid-leukotriens cascade and milk protein gene expression through an unknown mechanism. Colchicine and other tubulin binding drugs inhibit casein gene expression and DNA synthesis stimulated by prolactin whereas chemical compounds which alter microtubule without binding tubulin exert no inhibitory effect. Myo-inositol which suppresses some of the colchicine actions in several biological systems does not restore prolactin action after an inhibition by the drug. These data suggest that a tubulin molecule in the vicinity of the prolactin receptor, rather than actual microtubules, is involved in the transduction of the prolactin message from its receptor to milk protein genes.

prolactin / casein synthesis / tubulin / microtubule / colchicine / transduction

Résumé — Le rôle de la tubuline dans la transduction du message prolactinique. *La prolactine a beaucoup de fonctions biologiques différentes. Elle stimule la multiplication des cellules Nb_2 en empruntant le système de transduction par l'intermédiaire de la kinase C, elle stimule la sécrétion des protéines du lait par la voie phospholipase A_2-acide arachidonique-leucotriènes et elle stimule l'expression des gènes des protéines du lait et la synthèse de l'ADN dans la cellule mammaire par un mécanisme inconnu. La colchicine et un certain nombre d'autres drogues liant la tubuline s'opposent aux effets de la prolactine tandis que d'autres composés chimiques destabilisant les microtubules sans se lier à la tubuline ne s'y opposent pas. Le myo-inositol qui, dans plusieurs systèmes biologiques, supprime certains effets inhibiteurs de la colchicine, n'est pas capable de restaurer l'action stimulatrice de la prolactine en présence de la drogue. Ces faits suggèrent qu'une tubuline membranaire voisine du récepteur de la prolactine, plutôt que les structures microtubulaires proprement dites, est impliquée dans la transduction du message prolactinique.*

prolactine / synthèse de caséine / tubuline / microtubule / colchicine / transduction

INTRODUCTION

Prolactin has many known functions (Nicoll, 1980). Its mechanism of action is studied in only a limited number of tissues for a limited number of actions including:

— cell multiplication (Russell *et al,* 1987) (with mainly Nb_2 cells as experimental model);

— stimulation of gene expression (Devinoy *et al,* 1988) (with essentially milk protein gene as target genes);

— stimulation of protein secretion (Ollivier-Bousquet, 1984) (with mammary cell and milk secretion as an experimental model).

The prolactin receptor has been identified and recent studies have led to the cloning of its cDNA (Boutin *et al,* 1988). Transduction of the prolactin message beyond the receptor is mediated through an unknown mechanism which may or may not be a single mechanism. In the case of liver cells, nuclear kinase C is directly stimulated by prolactin added to isolated nuclei (Buckley *et al,* 1988). Nb_2 cell multiplication is stimulated by prolactin with a coincident increase of kinase C (Russell *et al,* 1987). The mechanism which transfers the prolactin message from its receptor to kinase C is unknown. Stimulation of milk secretion by prolactin is inhibited by various inhibitors of the phospholipids-arachidonic acid-leukotriens pathway and phospholipase A_2 mimic this prolactin action (Ollivier-Bousquet, 1984). The molecular mechanism between prolactin receptor and phospholipase A_2 remains unknown. In the case of casein gene expression, it is clear that a transduction mechanism is involved at the receptor level since anti-prolactin receptor antibodies mimic prolactin for the induction of casein synthesis (Djiane *et al,* 1981). Interestingly, prolactin stimulates DNA synthesis in cultured mammary explants (Houdebine, 1980), an effect also mimicked by anti-prolactin receptor antibodies (Djiane *et al,* 1981).

Very little is known about the molecular events which transfer the prolactin message from its receptor to milk protein genes (Houdebine *et al,* 1985; Devinoy *et al,* 1988, 1989). Studies carried out for several years have shown that colchicine and other drugs totally inhibit the prolactin stimulation of casein and DNA synthesis. The present paper summarizes these data and reveals some new experimental results.

MATERIALS AND METHODS

All the methods have been described in previous papers. Culture of mammary explants was carried out in serum-free medium in the presence of hormones. β-casein synthesis was estimated with a specific radioimmunoassay and β-casein mRNA concentration was measured using a specific cDNA probe (Zwierzchowski *et al,* 1987). Isolated epithelial mammary cells were cultured on floating collagen gel as previously described. β-casein was then measured in the cultured medium (Servely *et al,* 1987).

RESULTS

Effect of colchicine and other tubulin binding drugs on casein gene expression

Colchicine added at a millimolar concentration totally inhibits the induction of casein synthesis by prolactin (Houdebine and Djiane, 1980). This effect does not profoundly affect the prolactin receptor which remains down-regulated by the hormone (Djiane *et al,* 1980). This inhibition is also observed when prolactin is present in the culture medium without its major amplifiers, insulin and cortisol (Servely *et al,* 1987). Prolactin stimulated both casein synthesis and total protein synthesis. Colchicine inhibits both effects (Houdebine and Djiane, 1980).

Several other drugs known to disrupt microtubules after binding tubulin (vinblastin, podophyllotoxin, nocodazole, colcemide, tubulozole C) also strongly inhibit the induction of casein synthesis (Houdebine, 1980; Servely *et al,* 1987) whereas colchicine analogues (lumicolchicine, trimethylcolchicinic acid and colchicine) which do not alter microtubule structure do not prevent prolactin from acting (fig 1). The active drugs do not only affect casein secretion. Rather, they have a profound effect on prolactin action since β-casein mRNA accumulation is simultaneously blocked (Servely *et al,* 1987).

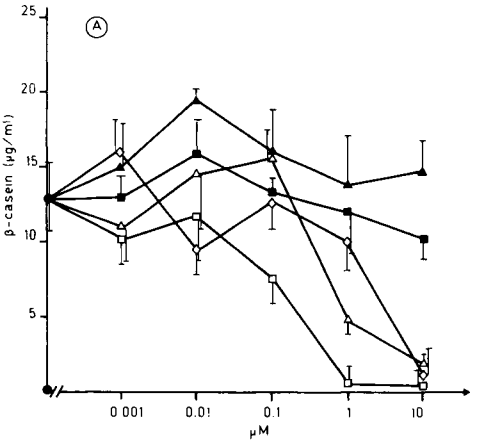

Fig 1. Effect of several tubulin binding drugs on the induction of β-casein synthesis in rabbit mammary cells. Cells were cultured for 3 d and then induced for 3 d in the presence of prolactin, insulin and cortisol with or without drugs. β-casein in the medium was measured at the end of the culture. (●—●) insulin + cortisol; (○—○) insulin (I) + cortisol (C) + prolactin (PRL); (□—□) I + C + PRL + colchicine; (■—■) I + C + PRL + lumicolchicine; (◊—◊) I + C + PRL + nocodazole; (Δ—Δ) I + C + PRL + tubulozole C; (▲—▲) I + C + PRL + tubulozole T.

Effect of drugs which disrupt microtubule without binding tubulin on casein gene expression

Several chemical compounds are known to alter microtubules although they do not bind tubulin. Griseofulvin and estramustine are potent microtubule disrupting drugs acting by binding microtubule associated proteins. When added to the culture medium of mammary explants, these drugs profoundly alter microtubule integrity yet they do not prevent prolactin from inducing casein synthesis (Houdebine *et al,* 1982; Zwierzchowski *et al,* 1987).

Similarly, several local anesthetics, procaine, tetracaine and dibucaine do not alter prolactin action on casein gene expression, although they markedly modify cytoskeleton structure (Houdebine *et al,* 1981).

Kinetics of colchicine action on casein gene expression

Colchicine and related drugs are considered to act rapidly on microtubules, and phenomena such as secretion are quickly affected. This is also the case for casein secretion which is markedly and rapidly inhibited by colchicine (Ollivier-Bousquet and Denamur, 1973). Surprisingly, the effect of colchicine, vinblastin, podophyllotoxin, nocodazole and gossipol on β-casein mRNA accumulation is not observed after a period as long as 8 h, whereas it is unambiguously seen after 24 h (Devinoy *et al,* 1988).

Taxol is known to prevent disruption of microtubule induced by colchicine. This compound added with several tubulin binding drugs is unable to completely restore prolactin stimulation (Devinoy *et al,* 1988). This fact must however be interpreted with

caution, since taxol *per se* attenuates prolactin effect.

Effect of tubulin binding drugs on DNA synthesis

The above-mentioned drugs which inhibit prolactin action on casein synthesis are all capable of also inhibiting the hormonal effect on DNA synthesis (Houdebine, 1980; Houdebine and Djiane, 1980). On the contrary, the drugs which do not affect casein synthesis induction are ineffective in preventing DNA synthesis stimulation by prolactin (Houdebine *et al,* 1981, 1982; Zwierzchowski, 1987).

Interestingly and unexpectedly, the tubulin binding drugs are capable of inhibiting not only the stimulation of DNA synthesis by prolactin, but also the stimulation by insulin and EGF (Martel and Houdebine, 1982).

Not surprisingly however, the prolactin-like effect of anti-prolactin receptor antibodies on DNA synthesis was totally suppressed by the tubulin binding drugs (Djiane *et al,* 1981).

The possible relation between colchicine and phospholipase C

In several reports, it is mentioned that colchicine strongly inhibits the phospholipase C which hydrolyses phosphatidylinositides (Schellenberg and Gillespie, 1977).

Several experiments not depicted here suggest that this type of phospholipase C mimics or stimulates a somewhat prolactin action on β-casein mRNA accumulation. These experiments cannot be reproduced in all cases and it is therefore unclear if phospholipase C is involved in prolactin action. More convincing is the fact that neomycin, a potent inhibitor of phosphatidylinositides hydrolysis (Carney *et al,* 1985), totally abrogates β-casein accumulation induced by prolactin (fig 2). However, neomycin is known to have other inhibitory effects and the possible involvement of phospholipase C in prolactin action cannot be ascertained from the only experiments described here.

In several biological systems, colchicine strongly inhibits phospholipase C and this inhibition is alleviated by an excess of myo-inositol but not by its analogue epi-inositol (Murray *et al,* 1951; Lymberopolous and Hawthorne, 1980). On the other hand, myo-inositol may circumvent a lack of phosphatidylinositol (Rodriguez *et al,* 1987).

Myo-inositol does not mimic or alter prolactin action and it is unable to alleviate the inhibitory effect of colchicine on β-casein mRNA accumulation (fig 2). These data suggest that colchicine inhibitory effect is not mediated through an inhibition of phospholipase C.

CONCLUSION

The data reported in the present paper argue strongly in favour of the idea that the integrity of tubulin is required for the transduction of the prolactin message from its receptor to milk protein genes. The fact that several drugs which destabilize the microtubular network without binding directly to tubulin, support the view that it is tubulin *per se,* rather than actual microtubules, which is involved in prolactin action. Tubulin has several molecular isoforms and at least 1 of them contains a structure which anchors the molecule in membranes. Tubulin or colchicine binding molecules have been found in several tissues including the mammary gland (Feit and Barondes, 1970; Lagnado *et al,* 1971; Stadler and Franke,

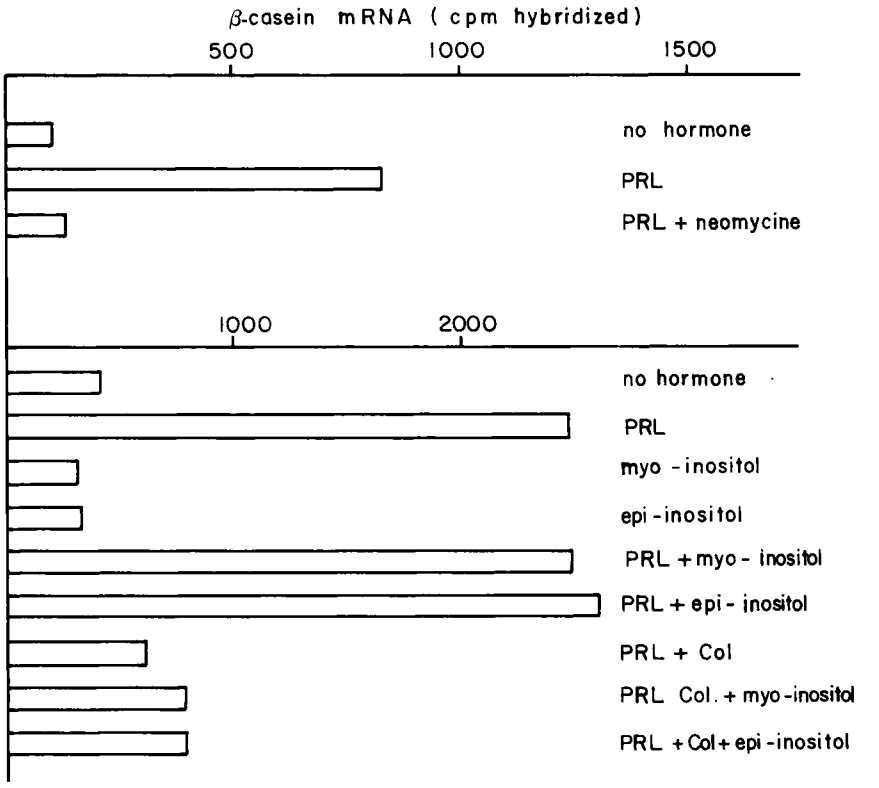

Fig 2. A Inhibition of β-casein mRNA accumulation by neomycin. The antibiotics were added with prolactin and β-casein mRNA was evaluated 8 hours after the beginning of the stimulation. **B** Effect of myo-inositol on the inhibition of β-casein mRNA accumulation. Mammary explants were cultured for 1 d in the presence of prolactin 1 μg/ml with or without colchicine (1 mmol/l). Casein mRNA was measured at the end of the culture.

1972, 1974; Bhattacharyya and Wolff, 1975, 1976; Houdebine *et al,* 1982). It is tempting to speculate that a tubulin molecule not recruited in a microtubular structure but anchored in the membranes in the vicinity of the prolactin receptor is involved in the transduction of the hormonal signal. Interestingly, the possible involvement of tubulin in the transmission of other signals through membranes has been reported (Wang *et al,* 1975; Chen *et al,* 1976; Gunther *et al,* 1976; Marks *et al,* 1978; Zor *et al,* 1978; Marks *et al,* 1980; Simonin *et al,* 1981; Carter *et al,* 1989; Ravindra *et al,* 1989). The fact that phospholipids were found to be associated with microtubular proteins, including tubulin (Hargreaves and McLean, 1988) further supports this view.

It is not clear how tubulin might interfere with the prolactin mechanism of action. It is reasonable to rule out the idea that cell multiplication is involved in this process. Indeed, at mid-pregnancy, rabbit mammary cells can differentiate without any previous cell multiplication and experimentally in the

presence of hydroxy-urea which blocks DNA synthesis (Houdebine and Djiane, 1980). The fact that stimulation of milk protein gene expression and DNA synthesis by prolactin are simultaneously inhibited strongly argues in favour of the idea that the prolactin mechanism of action is interrupted in one of its early steps, most likely at the membrane level after the binding of the hormone to its receptor. The observation that the insulin and EGF signals for DNA are also blocked by colchicine suggests that tubulin is functionally linked in some way to several membrane receptors. Experiments not shown here and based on the use of antitubulin antibodies to block tubulin action at the cellular level did not give unambiguous information. Indeed, these antibodies were unable to alter prolactin action on casein gene expression. The antibodies may have been unable to reach the membrane tubulin, and these experiments must be considered as inconclusive rather than arguing against the direct participation of membrane tubulin in prolactin action. Experiments which are in progress using primary mammary cells in culture should determine if colchicine prevents prolactin from stimulating casein gene transcription. If this happens to be the case, these experiments will bring a strong argument in favour of the hypothesis that it is the whole prolactin message which is blocked by the anti-tubulin drugs at the membrane level.

Colchicine and related drugs have been shown to markedly decrease the uptake of ovine prolactin into the liver Golgi light and intermediate fractions (Posner *et al,* 1982). This observation and the data reported here support the view that the endocytosis of the prolactin-receptor complex is required for the hormone to deliver its message to milk protein genes.

The fact that colchicine and related drugs act at the membrane level does not eliminate the idea that microtubule or more generally cytoskeleton is an essential structure in mammary cell differentiation. Indeed, cytochalasin D (Blum and Wicha, 1988) although not cytochalasin B (Houdebine and Djiane, 1980) which disrupts microfilaments, prevents milk protein mRNA from accumulating without altering transcription level of these genes (Wicha *et al,* unpublished data). It is therefore the half-life of milk protein mRNA which is then affected rather than the transmission of the hormonal message to genes. The fact that milk protein mRNA were found to be associated with cytoskeleton further supports this view (Aggeler *et al,* unpublished data). More generally, it is the whole cell structure which seems to be involved in the transmission of the hormonal message and in the differentiation process of the mammary cell (Aggeler *et al,* 1988). The observation that the extracellular matrix, which is known to play an essential role in mammary cell polarization and differentiation, enhances milk protein mRNA concentration without any simultaneous increase of milk protein gene transcription (Eisenstein and Rosen, 1988) brings additional support to this idea.

It is not known if colchicine and related drugs are also able to block the prolactin signal triggering milk secretion, and this stimulation of Nb_2 cell multiplication. It is not easy to evaluate the effect of colchicine on the stimulation of secretion since the drug is *per se* a strong blocker of the secretion process. To the best of our knowledge, the effect of colchicine on the stimulation of DNA synthesis by prolactin in Nb_2 cells has not been examined.

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