Virchows Archiv (1994) 425:467–472

ORIGINAL ARTICLE

Paola Cassoni · Anna Sapino · Francesco Negro
Gianni Bussolati

Oxytocin inhibits proliferation of human breast cancer cell lines

Received: 23 June 1994/Accepted: 13 September 1994

Abstract In this study we show that treatment of MDA-MB231 hormone-independent human breast cancer cells with oxytocin (OT) or with the OT analogue F314 induces significant growth inhibition together with a change in cell phenotype. In MCF7 and T47D human breast cancer cells, OT inhibits oestrogen-induced cell growth. In these same cells, OT administration significantly enhances the inhibitory effect of tamoxifen on cell proliferation. MDA-MB231, MCF7 and T47D cells all express mRNA specific for the OT receptor. These data suggest that it may be possible to inhibit breast cancer growth using OT and OT analogues.

Key words Oxytocin · Breast cancer · Cell growth

Introduction

We recently reported that administration of the neurohypophyseal peptide oxytocin (OT) produces a relative increase in myoepithelial cell number in oestrogen-treated mouse mammary gland, both in vivo and in organotypic cultures [18]. This was paralleled by a lesser increase in luminal cell number while the number of undifferentiated cells decreased significantly. We therefore interpreted these findings as evidence that OT, in addition to the established effect on cell contraction, can influence cell growth and induce stem cell differentiation.

We tested the hypothesis that OT and OT analogues (synthetic peptides binding to OT receptors but devoid of contractile activity) may exert the same effect on the growth of breast cancer cells. To this end we performed experiments on both oestrogen-dependent and oestrogen-independent human breast cancer cell lines.

P. Cassoni · A. Sapino · F. Negro · G. Bussolati (✉)
Department of Biomedical Sciences and Oncology,
University of Turin, Via Santena 7,
I-10126 Turin, Italy

Materials and methods

Reagents

OT, kindly supplied by Ferring Research Institute (Malmo, Sweden), was used at final concentrations of 10^{-7}, 10^{-8} and 10^{-9} M. OT analogue F314 [Mpa[1], D-Tyr(Et)[2], Thr[4], Orn[8]-OT] (Ferring) was used at concentrations of 10^{-8} and 5×10^{-8} M. Both substances were dissolved in distilled water. When required, 17β-oestradiol (E_2) (Sigma, St. Louis, Mo., USA) was added to the cultures in order to obtain a final concentration of 10^{-8} M. Tamoxifen (TAM) (*trans-P*-dimethylaminoethoxyphenyl-1-1,2 diphenylbut-1-en, ICI 46474) (ICI Pharmaceuticals, Macclesfield, UK) was used at a final concentration of 10^{-6} M.

Cell lines

The human breast cancer cell lines MCF7 and T47D (oestrogen-dependent) and MDA-MB231 (oestrogen-independent), and the HT29 colon carcinoma cells were obtained from the American Type Culture Collection (Rockville, Md., USA) [4, 8, 22]. T47D, MDA-MB231 and HT29 cells were routinely cultured in RPMI (Gibco, Burlington, Ontario, Canada) supplemented with 10% fetal calf serum (FCS) (Gibco). MCF7 cells were grown in DMEM-F12 (Sigma) added at low (5%) or high (10%) FCS concentrations.

Cells were grown in Petri dishes at 37° C in humidified air/5% CO_2 atmosphere.

Studies on cell growth

Cells were seeded in triplicate in 24-multiwell plates at a density ranging from 15,000 to 40,000 cells/ml in basal medium. OT or OT analogue F314 were added at the previously indicated concentrations 48 h after plating. The medium was changed every 24 h.

The effect of OT on the hormone-dependent MCF7 and T47D cell lines was evaluated with or without E_2 (10^{-8} M) or TAM (10^{-6} M), in order to determine the existence of an OT interaction with these substances. For the growth curves, cells were removed at 48, 96 and 144 h of culture using 500 µl trypsin-EDTA solution (Gibco)/well and counted double blind by two independent investigators using a Coulter counter and a haemocromocytometer. Statistical analysis on growth curves was carried out using one-way analysis of variance (ANOVA).

In some experiments, the growth fraction was evaluated in ELISA by 5-bromo-2′-deoxyuridine (BrdU) incorporation (BrdU Labelling and Detection Kit, Boehringer, Mannheim, Germany) at 48 and 96 h of culture. Cell proliferation was expressed as extinc-

Table 1 Specific cytoplasmic markers

Marker	Reagent	Source	Dilution
Cytokeratin (19, 16, 15, 14, 10)	AE1 (mAb)	Cambridge Research (Cambridge, Mass.)	1:10
Cytokeratin (18, 8, 7, 6, 5, 4, 3, 2, 1)	AE3 (mAb)	Cambridge Research	1:1
Cytokeratin (8)	35βH11 (mAb)	Enzo (New York)	1:20
Cytokeratin (14, 10, 5, 1)	34βE12 (mAb)	Enzo	1:20
α-Smooth muscle actin	1A4 (mAb)	Sigma (St. Louis, Mo.)	1:20
Collagen type IV	Antiserum	Heyl (Berlin, Germany)	1:10

tion of the sample, determined using a microtitre plate reader at 405 nm. Statistical analysis was carried out by ANOVA.

Immunofluorescence

For immunocytochemical tests, in a standard indirect immunofluorescence procedure [15, 17, 19] both untreated and treated MDA-MB231 and MCF7 cells were grown on glass coverslips for 5 days. After washing in phosphate buffered saline (PBS), cells were fixed in methanol for 5 min at –20° C, permeabilized in acetone for 5 s at –20° C, and rehydrated with pig normal serum (diluted 1:50 in PBS for 20 min). Cells were incubated at 37° C for 60 min with different primary monoclonal antibodies listed in Table 1. The appropriate fluorescein-labelled secondary antiserum (Sera-Lab, Sussex, UK) diluted 1:10 in PBS was used for 30 min at room temperature.

Detection of OT receptor mRNA

For OT receptor mRNA detection, total cell RNA was extracted by the guanidinium-isothiocyanate procedure from cultured cells brought to confluence [5]. Total RNA (10 μg) was subjected to reverse transcription (RT) and polymerase chain reaction (PCR) amplification according to a previously published procedure [24]. Amplified DNA was electrophoresed on a 1.2% agarose gel and directly stained with ethidiumbromide, or transferred to nylon membrane [12] and hybridized to a ^{32}P end-labelled synthetic 48-bp oligonucleotide probe [24]. As a positive control we used total RNA extracted from the uterus of a patient who had undergone hysterectomy after uncontrollable uterine bleeding at term.

Results

1) Effect of OT and of the OT analogue F314 on cell growth

The effect of OT on cell proliferation was determined in the breast cancer cell lines and, in parallel, in the colon carcinoma cell line HT29.

The growth of human colon cancer HT29 cells was not affected by the addition of OT 10^{-8} and 10^{-7} M (Fig. 1A). Proliferation of MDA-MB231 cells was rapidly and increasingly reduced by OT 10^{-8} M and 10^{-7} M (Fig. 1B). OT 10^{-8} M and 10^{-7} M respectively induced a 27% and a 34% inhibition of cell growth at 144 h. The significant inhibitory effect of OT on cell proliferation was confirmed by the BrdU incorporation assay (proliferation expressed as extinction of the samples; MDA-MB231 untreated vs 10^{-7} M OT treated at 48 h: 0.130±0.004 vs 0.079±0.007, P=0.004; at 96 h: 0.206±0.009 vs 0.143±0.007, P=0.018). At a concentration of 10^{-9} M OT did not show any significant effect on MDA-MB231 cell growth (Fig. 1B).

The OT analogue F314 (10^{-8} M and 5×10^{-8} M) caused a strong and significant inhibition of MDA-MB231 cell growth at all time points (44% at 144 h) (Fig. 1C).

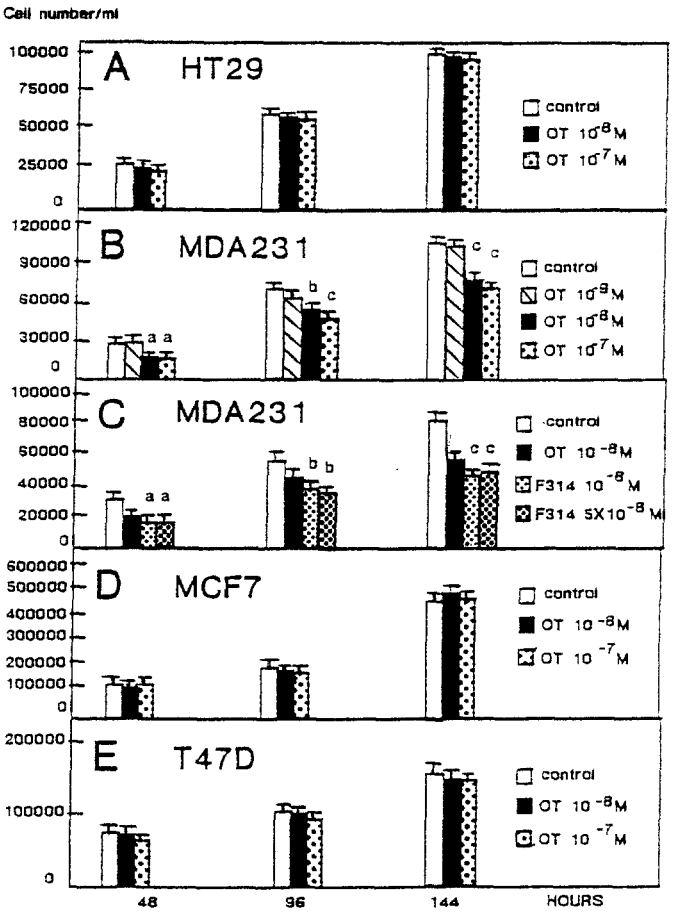

Fig. 1A–E Effect of oxytocin (OT) on HT29, MDA-MB231 and MCF7 cell growth in basal conditions of culture. **A** HT29 colon cancer cell growth is not affected by treatment with OT 10^{-8} and 10^{-7} M. **B** MDA-MB231 proliferation is strongly inhibited by OT 10^{-8} M and 10^{-7} M at each time-point (OT 10^{-8} M and 10^{-7} M vs control: [a] P=0.03 at 48 h and [c] P=0.0001 at 144 h; at 96 h of treatment OT 10^{-8} M vs control: [b] P=0.001; OT 10^{-7} M vs control: [c] P=0.0001). OT 10^{-9} M has no significant effect on cell growth. **C** OT analogue F314 10^{-8} M and 5×10^{-8} M inhibits MDA-MB231 proliferation at all time-points ([a] P=0.01 at 48 h, [b] P=0.001 at 96 h; [c] P=0.0001 at 144 h vs control). **D, E** MCF7 and T47D cell growth in basal conditions of culture is not modified by addition of OT. Data are mean and SD from three separate experiments done in triplicate. Statistical analysis carried out by ANOVA

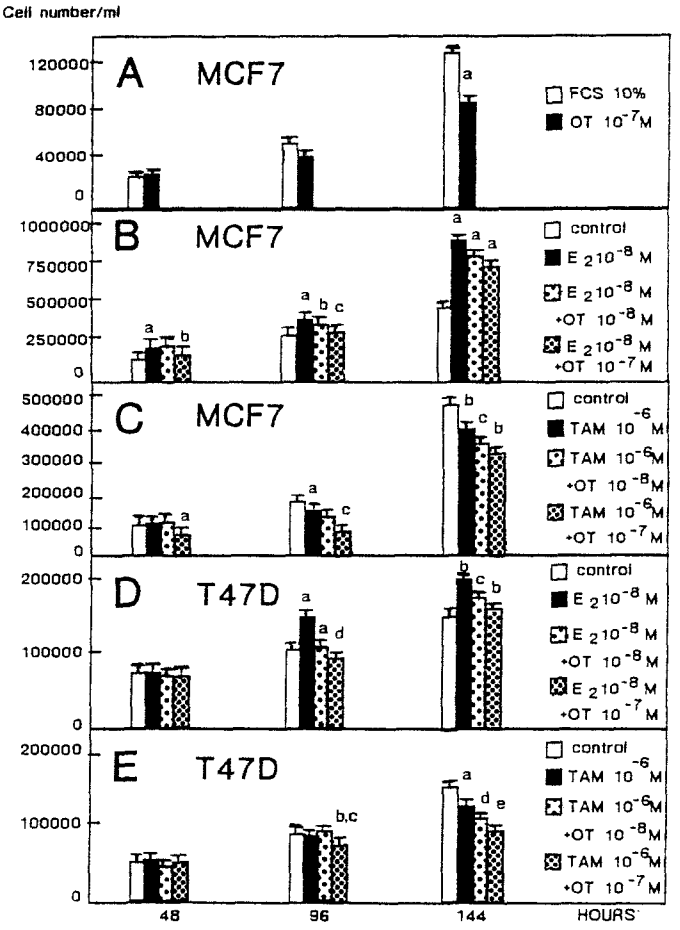

Fig. 2A–E Effect of OT on MCF7 and T47D cell proliferation in the presence of high concentrations of fetal calf serum (FCS) and in the presence of 17β-oestradiol (E_2) or tamoxifen (TAM). **A** In the presence of high (10%) FCS concentration OT 10^{-7} M exerts a negative effect on cell proliferation, which becomes significant at 144 h of treatment (^{a} P=0.01). **B** E_2 10^{-8} M significantly enhances MCF7 growth at 48, 96 and 144 h of culture (^{a} P=0.0001 vs control). Addition of OT 10^{-8} M to oestrogen-enriched medium reduces the steroid-induced proliferation at 96 h (^{b} P=0.003 versus E_2 alone) and 144 h (^{a} P=0.0001 vs E_2 alone). Inhibiting effect of OT 10^{-7} M is significant at all times (E_2 vs E_2+OT 10^{-7} M: ^{b} P=0.003 at 48 h, ^{c} P=0.009 at 96 h and ^{a} P=0.0001 at 144 h). **C** TAM 10^{-6} M significantly reduces MCF7 proliferation at 96 h (^{a} P=0.009 vs control) and 144 h of culture (^{b} P=0.0001 vs control). OT 10^{-8} M enhances the effect of TAM alone at 144 h (^{c} P=0.01). OT 10^{-7} M causes a strong additive effect on TAM growth control at 48 h (^{a} P=0.009), 96 h (^{c} P=0.01) and 144 h (^{b} P=0.0001) when compared to TAM alone. **D** The T47D cell proliferation is enhanced by E_2 10^{-8} M at 96 h (^{a} P=0.02 vs control) and 144 h (^{b} P=0.0001 vs control) of culture. OT 10^{-8} M significantly reduces the effect of E_2 alone at 96 h (^{a} P=0.02) and 144 h (^{c} P=0.017, E_2 vs E_2+OT 10^{-8} M). OT 10^{-7} M exerts a similar effect (^{d} P=0.012 at 96 h and ^{b} P=0.0001 at 144 h, E_2 vs E_2+OT 10^{-7} M). **E** TAM 10^{-6} M significantly inhibits T47D cell growth after 144 h of culture (^{a} P=0.015 vs control). When OT 10^{-7} M is added to TAM the inhibitory effect is significant at 96 h of incubation (^{b} P=0.005 vs control and ^{c} P=0.007 vs TAM alone). At 144 M both OT 10^{-8} h and 10^{-7} M enhance the effect of TAM alone (^{d} P=0.012, TAM vs TAM+OT 10^{-8} M and ^{e} P=0.0001, TAM vs TAM+OT 10^{-7} M). Data are mean and SD from three separate experiments done in triplicate. Statistical analysis carried out by ANOVA

The growth of hormone-dependent MCF7 and T47D breast cancer cell lines was not affected by the addition of OT 10^{-8} and 10^{-7} M to standard medium (Fig. 1D, E). However, at high concentrations (10%) of FCS, OT 10^{-7} M significantly reduced MCF7 proliferation at 144 h of culture (32% of growth inhibition, P=0.01), but not at 48 and 96 h (Fig. 2A).

Incubation of MCF7 and T47D cells in the presence of E_2 10^{-8} M resulted in a significant increase of cell proliferation at 96 and 144 h of culture (Fig. 2B, D). When OT 10^{-8} and 10^{-7} M was added to E_2-enriched medium, the mitogenic effect of oestradiol was significantly reduced (22% reduction for MCF7 and 27% for T47D at 144 h with OT 10^{-7} M) (Fig. 2B, D).

Treatment of MCF7 cells with TAM 10^{-6} M induced a growth inhibition which was enhanced by the simultaneous addition of OT 10^{-7} M at all time points (23% increment in TAM-induced growth inhibition at 144 h, P=0.0001) (Fig. 2C). Addition of OT 10^{-8} M to TAM-treated MCF7 cells significantly increased the inhibitory effect only after 144 h of incubation (Fig. 2C).

Similarly, the anti-proliferatory effect of TAM on T47D cells was significantly enhanced by the addition of OT 10^{-8} and 10^{-7} M (30% increment of growth inhibition at 144 h) (Fig. 2E).

2) Immunofluorescence

Using immunofluorescent techniques MDA-MB231 cells stained positively with all the antibodies recognizing the different cytokeratins. Cytoskeletal filaments appeared evenly distributed in the cytoplasm and along cell processes. Treatment with OT resulted in a marked cytoskeletal rearrangement in most cells, since the cytokeratin filaments almost disappeared from the processes, forming instead a dense paranuclear whirl. This was especially marked for keratins of the basal type recognized by monoclonal 34βE12 (Fig. 3A, B).

MCF7 cells cultured in 10% FCS both in controls and following OT treatment were negative for monoclonal antibodies 34βE12 and 35βH11 while AE1 antibody, recognizing acidic keratins, revealed a diffuse meshwork of cytoskeletal filament. AE3 monoclonal antibody in control cells gave a faint granular staining, while in treated cells the staining was definite and arranged in organized filaments (Fig. 3C, D).

Staining with anti-α smooth muscle actin antibody was uniformly negative in all cell types. Collagen type IV was expressed in MDA-MB231 cells (both in test and controls) while negative in MCF7 cells.

3) Detection of OT receptor mRNA by RT-PCR

An amplified band of 391 bp corresponding to a region of the OT receptor cDNA was detected after RT-PCR of equal amounts of RNA extracted from MCF7, T47D and MDA-MB231 cells (Fig. 4). Conversely, no signal was

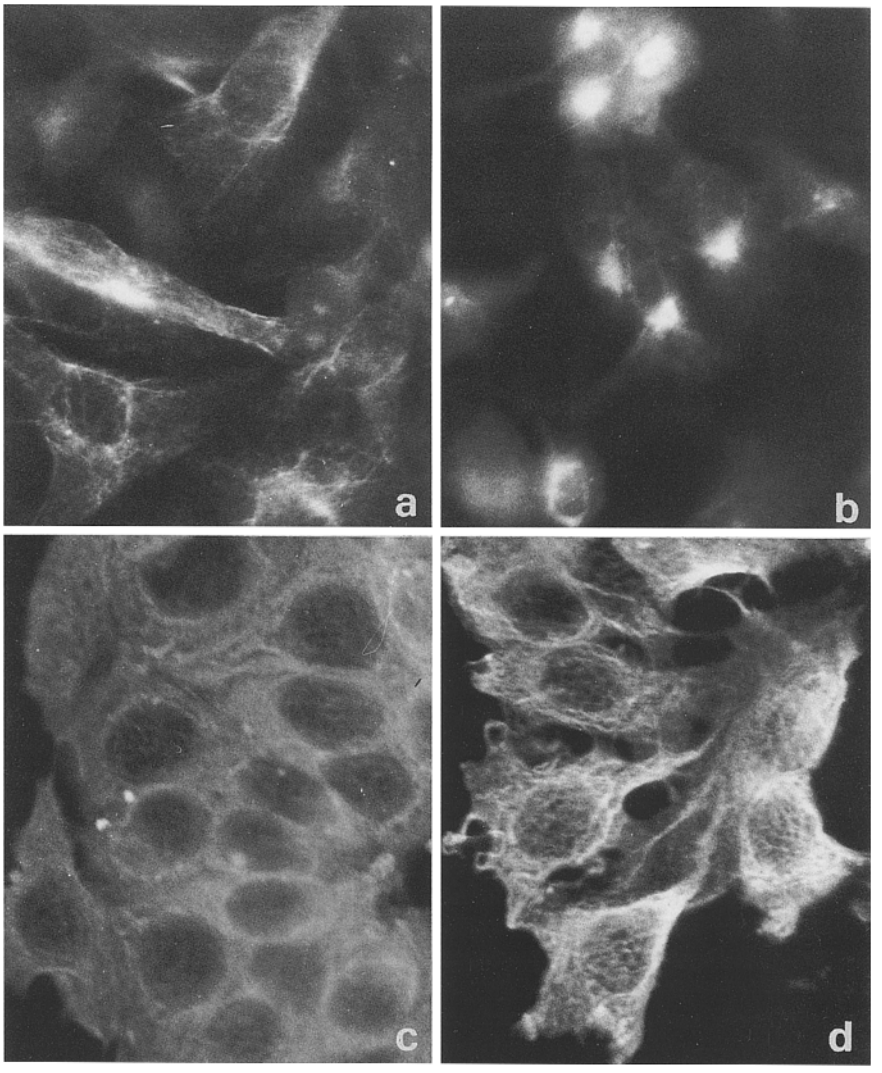

Fig. 3a–d Cytoskeletal structures in MDA-MB231 and MCF7 cells. **a** Immunofluorescence for cytokeratin (34βE12 antibody) shows a diffuse cytoplasmic distribution of filaments in untreated MDA-MB231 cells which appear spindle-shaped. **b** Cells treated with OT 10^{-7} M for 5 days show a rearrangement of keratin filaments, which are mostly localized in dense paranuclear areas or whirls. **c** MCF7 cells (untreated) are poorly reactive with AE3 monoclonal antibody against basic keratins. **d** In MCF7 cells treated with OT 10^{-7} M for 5 days, immunofluorescence shows an extensive and diffuse array of cytoskeletal filaments

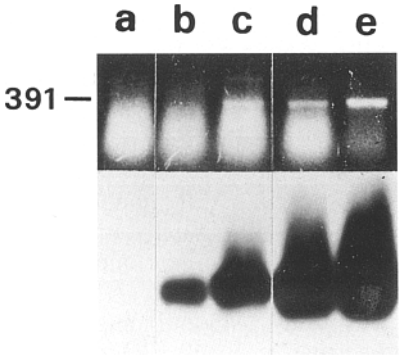

Fig. 4 Detection of OTr mRNA by reverse transcription polymerase chain reaction. Amplified DNA was run on a 1.2% agarose gel and stained with ethidium bromide (*top*) or transferred to nylon membrane and hybridized to a ^{32}P oligonucleotide probe (*bottom*). *Lanes a–e* show the DNA obtained from cell lines HT29 (*a* 20 µg total RNA), T47D (*b* 10 µg), MDA-MB231 (*c* 10 µg) and MCF7 (*d* 10 µg) and from uterus (*e* 2 µg)

detected even after gel-blot hybridization with a specific probe when RNA from HT29 cells was used for amplification. RT-PCR of RNA extracted from uterus at term was used as a positive control.

Discussion

The present study confirms our previous data on a role for OT in the control of mammary cell growth [18]. In fact, this peptide markedly reduced the cell proliferation rate in oestrogen-independent MDA-MB231 cells as well as in oestrogen-dependent MCF7 and T47D cells, as demonstrated by both cell counting and BrdU uptake.

The observed decrease in growth rate was paralleled by a change in immunophenotype, possibly suggesting differentiation. In fact, as a result of exposure to OT, in MDA-MB231 cells cytokeratins formed a dense whirl mostly localized in paranuclear areas while in the MCF7 cells OT induced a structural arrangement of basic keratins recognized by AE3 monoclonal antibody, with building up of cytoplasmic filaments. Cytokeratins 5 and

14, recognized by monoclonal antibodies AE3 and 34βE12, are known to be expressed in myoepithelial cells while absent in most breast carcinomas [1, 2].

Taken together, the main changes in immunophenotype following OT treatment are therefore enhanced expression of basic keratins paralleled by a cytoskeletal rearrangement. We previously demonstrated similar structural changes, linked to an inhibition of proliferation, in oestrogen-deprived MCF7 cells [15]. In our previous observations on the developing mouse mammary gland OT, in combination with other mammotrophic hormones, induced cell differentiation, marked by an increase in the relative number of myoepithelial and luminal epithelial cells and by a decrease of undifferentiated "null" cells [18]. This would suggest that malignant cells could be likened to the undifferentiated stem cells which are detectable, as a small minority, within the population of the normal mammary gland. Analogies between breast cancer cells and mammary stem cells had already been proposed on the basis of cell kinetics and immunocytochemical studies [13, 21].

The present findings on the effect of OT on breast cancer cells are in agreement with other evidence exemplifying how mammotrophic hormones can induce differentiation associated with a decreased proliferation rate. We have previously observed in organ cultures of mammary gland that prolactin (added to the medium at concentrations in the same range as those used here for OT) induced a marked secretory activity in the alveolar cells, while there was a net reduction of BrdU-positive proliferating nuclei [16].

Olins and Bremel [11] have shown that OT exerts a double action on target cells: it activates contraction through myosin phosphorylation, and causes an increase in cytoplasmic cAMP [11]. However, while cell contraction is stimulated by OT concentrations in the range of 10^{-9} M, the level of intracytoplasmic cAMP is only affected by OT concentrations in the range of 10^{-8} or 10^{-7} M or higher [20].

Interestingly, in our experiments on breast cancer cell lines growth inhibition was obtained only at relatively high doses of OT. This suggests that this effect may be related to a dose-dependent increase in cytoplasmic cAMP induced by OT. In fact a similar effect can be induced in breast cancer cells by cAMP analogues [23]. It is well known that cAMP acts on cell growth inhibiting c-*ras* and c-*myc* protein expression through a modulation of protein kinase A [7]. Furthermore, it has recently been shown that cAMP may negatively modulate growth by blocking transmission of signals from *ras* to *raf* 1, thereby preventing activation of the MAP kinase cascade [6, 26]. We suggest that these mechanisms might be responsible for the reduction of mitotic activity in breast cancer cells induced by OT and by OT analogues. It is known that the latter can antagonize OT contractile activity, specifically binding to OT receptors [9, 10].

Taylor et al. [25] have previously reported a significant enhancement of the growth rate of MCF7 cells cultured in DMEM medium with 2.5% FCS as a result of OT administration at concentrations ranging from 10^{-9} to 10^{-12} M. When we tested the effect of 10^{-9} M OT on the proliferation rate of oestrogen-dependent MCF7 and T47D breast cancer cell lines in basal conditions (5% FCS) we did not detect any significant response. In our experiments, in the presence of 10% FCS, OT concentrations at least two orders of magnitude higher than those used by Taylor et al. effectively inhibited cell growth rather than stimulating it. We suppose that a different oestrogen content in the culture medium (related to different FCS concentrations) might be partly responsible for the discrepancy. Steroid hormones are known to enhance the sensitivity of target cells to OT, possibly by modulating the expression of OT receptors [14, 20]. Therefore, high E_2 levels in the culture medium could increase the number of OT binding sites in MCF7 and T47D cells, amplifying the effect of the peptide on cell growth.

Previous works have suggested that regulation of growth-hormone-dependent rat mammary tumours and breast cancer cell lines depends on the antagonist action between oestrogens and cAMP [3]. This regulatory mechanism is distinct from that mediated by TAM, a well-known antioestrogenic agent, which unlike cAMP analogues does not affect c-*myc* or c-*ras* protein expression [7]. These data seem to be in agreement with a series of experiments where we tested the combined effect of TAM and OT. In these experimental conditions, OT enhanced the inhibitory effect of TAM on the MCF7 and T47D cells apparently through an additive effect.

Indirect evidence supporting the hypothesis that the observed inhibition of cell growth is related to activation of specific receptors comes from the demonstration, obtained by RT-PCR, of the presence of OT receptor mRNA in the responsive cell lines and not in control colon carcinoma cells. These findings, which represent the first evidence of the presence of OT receptor mRNA in breast carcinoma cells, are also in agreement with the prior observation of Taylor et al. [25] on the presence of OT binding sites in the MCF7 cells. We have preliminary data indicating that OT receptor mRNA is transcribed in cases of primary human breast cancer.

In conclusion, we describe here a receptor-mediated inhibitory effect of OT and OT analogues on the proliferation of human breast carcinoma cells, paralleled by a change in immunophenotype. In addition, we have observed that OT inhibits oestrogen-induced proliferation and has a synergic effect with TAM. These findings open new prospects in the field of breast cancer hormone responsiveness and control.

Acknowledgements This work was supported by grants from the Italian CNR (Rome) (grant no. 93.012125.PF39), AIRC (Milan) and the Ministry of the University. The authors wish to thank Prof. Carola Ponzetto (University of Turin) for critically reviewing the manuscript. Miss Vittoria Bravoco and Dr. Laura Dinolfo made a valuable contribution to the experiments. Mr. Nino Ferraro is thanked for this photographic assistance. Dr. Enrico Gottardi kindly synthesised the oligonucleotides. Samples of OT and OT analogue F314 were generously supplied by Dr. P. Melin (Ferring Research Institute, Sweden).

References

1. Böcker W, Bier B, Freytag G, Brommelkamp B, Jarash ED, Edel G, Dockhorn-Dworniczak B, Schmidt KW (1992) An immunohistochemical study of the breast using antibodies to basal and luminal keratins, alpha-smooth muscle actin, vimentin, collagen IV and laminin. I. Normal breast and benign proliferative lesions. Virchows Arch [A] 421:315–322
2. Böcker W, Bier B, Freytag G, Brommelkamp B, Jarash ED, Edel G, Dockhorn-Dworniczak B, Schmidt KW (1992) An immunohistochemical study of the breast using antibodies to basal and luminal keratins, alpha-smooth muscle actin, vimentin, collagen IV and laminin. II. Epitheliosis and ductal carcinoma in situ. Virchows Arch [A] 421:323–330
3. Bodwin JS, Clair T, Cho-Chung YS (1978) Inverse relation between estrogen receptors and cyclic adenosine 3′,5′-monophosphate-binding proteins in hormone-dependent mammary tumor regression due to dibutyryl cyclic adenosine 3′:5′-monophosphate treatment or ovariectomy. Cancer Res 38:3410–3413
4. Cailleau R, Young R, Olive' M, Reeves WJ (1974) Breast tumor cell lines from pleural effusions. J Natl Cancer Inst 53:661–674
5. Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-PhOH-chloroform extraction. Anal Biochem 162:156–159
6. Cook SJ, McCormick F (1993) Inhibition by cAMP of Ras-dependent activation of Raf. Science 262:1069–1072
7. Katsaros D, Ally S, Cho-Chung YS (1988) Site-selective cyclic AMP analogues are antagonistic to estrogen stimulation of growth and proto-oncogene expression in human breast cancer cells. Int J Cancer 41:863–867
8. Keydar I, Chen L, Weiss FR, Delarea J, Radu M, Chaitcik S, Brenner HJ (1979) Establishment and characterization of a cell line of human breast carcinoma origin. Eur J Cancer 15:659–670
9. Melin P, Vilhardt H, Lindeberg G, Larsson LE, Akerlund M (1981) Inhibitory effect of O-alkylated analogues of oxytocin and vasopressin on human and rat myometrial activity. J Endocrinol 88:173–180
10. Aurell CJ, Johansson E, Persson R, Berdén L, Nilsson A, Trojnar J, Abbe M, Ekholm K, Hansson C, Nylen A, Rosenquist A, Johansson B, Persson C, Melin P (1993) Synthesis and pharmacological properties of some Carba-6 and Carba-1 oxytocin antagonists. In: Schneider CH, Eberle AN (eds) Peptides 1992, ESCOM Science Publishers, Leiden, pp 697–699
11. Olins GM, Bremel RD (1984) Oxytocin stimulated myosin phosphorylation in mammary myoepithelial cells: roles of calcium ions and cyclic nucleotides. Endocrinology 114:1617–1626
12. Reed KC, Manor DA (1985) Rapid transfer of DNA from agarose gel to nylon membranes. Nucleic Acids Res 13:7207–7221
13. Russo J, Russo IH (1980) Influence of differentiation and cell kinetics on the susceptibility of the rat mammary gland to carcinogenesis. Cancer Res 40:2677–2681
14. Sala NL, Freire F (1974) Relationship between ultrastructure and response to oxytocin of the mammary myoepithelium throughout pregnancy and lactation: effect of estrogen and progesterone. Biol Reprod 11:7–17
15. Sapino A, Pietribiasi F, Bussolati G, Marchisio PC (1986) Estrogen- and tamoxifen-induced rearrangement of cytoskeletal and adhesion structures in breast cancer MCF-7 Cells. Cancer Res 46:2526–2531
16. Sapino A, Macri' L, Gugliotta P, Bussolati G (1990) Immunocytochemical identification of proliferating cell types in mouse mammary gland. J Histochem Cytochem 38:1541–1547
17. Sapino A, Papotti M, Sanfilippo B, Gugliotta P, Bussolati G (1992) Tumor types derived from epithelial and myoepithelial cell lines of R3230AC rat mammary carcinoma. Cancer Res 52:1553–1560
18. Sapino A, Macri' L, Tonda L, Bussolati G (1993) Oxytocin enhances myoepithelial cell differentiation and proliferation in the mouse mammary gland. Endocrinology 133:838–842
19. Sapino A, Macri' L, Gugliotta P, Pacchioni D, Liu YJ, Medina D, Bussolati G (1993) Immunophenotypic properties and estrogen dependency of budding cell structures in the developing mouse mammary gland. Differentiation 55:13–18
20. Soloff MS (1979) Regulation of oxytocin action at the receptor level (minireview). Life Sci 25:1453–1459
21. Sonnenberg A, Daams J, Van der Valk MA, Hilkens J, Hilgers J (1990) Development of the mouse mammary gland. Identification of stages in differentiation of luminal and myoepithelial cells using monoclonal antibodies and polyvalent antiserum against keratin. J Histochem Cytochem 34:1036–1046
22. Soule HD, Vazquez J, Long A, Albert S, Brennan M (1973). A human cell line from a pleural effusion derived from a breast carcinoma. J Natl Cancer Inst 51:1409–1416
23. Tagliaferri P, Katsaros D, Clair T, Ally S, Tortora G, Neckers L, Rubalcava B, Parandoosh Z, Chang Y, Revankar GR, Crabtree GW, Robins RK, Cho-Chung YS (1988) Synergistic inhibition of growth of breast and colon human cancer cell lines by site-selective cyclic AMP analogues. Cancer Res 48:1642–1650
24. Takemura M, Nomura S, Kimura T, Inoue T, Onoue H, Azuma C, Saji F, Kitamura Y, Tanizawa O (1993) Expression and localization of oxytocin receptor gene in human uterine endometrium in relation to the menstrual cycle. Endocrinology 132:1830–1835
25. Taylor AH, Ang VTY, Jenkins JS, Silverlight JJ, Coombes RC, Luqmani YA (1990) Interaction of vasopressin and oxytocin with human breast carcinoma cells. Cancer Res 50:7882–7886
26. Wu J, Dent P, Jelinek T, Wolfman A, Weber MJ, Sturgill TW (1993) Inhibition of the EGF-activated MAP kinase signaling pathway by adenosine 3′,5′-monophosphate. Science 262: 1065–1068