**in vivo** *24*: 157-164 (2010)

# Oxytocin Both Increases Proliferative Response of Peripheral Blood Lymphomonocytes to Phytohemagglutinin and Reverses Immunosuppressive Estrogen Activity

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**Abstract.** *Background: It has been shown that the neurohypophyseal peptide oxytocin is present in the human thymus and in vitro it can mimic interleukin (IL)-2 action in the induction of interferon-γ production. In the present study, we tested the capacity of oxytocin to modulate the response of peripheral blood mononuclear cells (PBMCs) to phytohemagglutinin (PHA) and its ability to change the membrane expression of IL-2 receptor CD25 and the CD95 activation marker. Furthermore, whether oxytocin was able to reverse the inhibition of PBMC blastic response and CD25 expression induced by estradiol benzoate ($E_2B$) was studied. Patients and Methods: Fifteen healthy women were studied with a mean age of 33.8 years, no previous pregnancies, all in the early follicular phase of the cycle with normal values of circulating estrogens. Results: The addition of oxytocin ($1\times10^{-10}$ M, $1\times10^{-11}$ M, $1\times10^{-12}$ M) significantly increased the PBMC blastic response to PHA as well as the expression of both CD25 and CD95. These results were due to interaction of oxytocin with its specific receptor since the addition of an oxytocin antagonist completely reversed the oxytocin activity. In contrast, $E_2B$ induced a marked decrease of PHA-stimulated PBMC cell cycle progression and CD25 expression: the inhibitory effect of $E_2B$ was significantly counteracted by low concentrations of oxytocin. Conclusion: The present results support the hypothesis that neuropeptides may act as a link in the network between the immune and the neuroendocrine systems.*

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*Key Words:* Oxytocin, estrogen, interleukin-2, lymphocytes, T-cell receptor, cell cycle.

The immune and neuroendocrine systems are two key components of mammalian organisms, with dynamic interactions in the execution of their designated functions. Both systems communicate through cytokines, neurotransmitters and hormones which act synergistically so that immunendocrine influences appear to be a part of single multidirectional circuitry. Physiologically, the thymus is under neuroendocrine control that appears to be extremely complex, with specific interplay of multiple factors involving the production of pituitary hormones and the action of systemic hormones, as well as the expression of their respective receptors on thymic cells. For example, it has been shown that the neurohypophyseal peptide oxytocin is present in the human thymus (1) and *in vitro* it can mimic interleukin (IL)-2 action in the induction of interferon-γ production (2). Furthermore, estrogens reduce thymus weight (3, 4), cause a transient reduction in plasma thymosine α-1, a peptide component of the partially purified thymic extract thymosin fraction 5 (5), and directly inhibit lymphocyte reactivity to mitogens such as phytohemagglutinin (PHA) (6, 7). These effects might be involved, to some extent, in the depression of the immune system usually observed during pregnancy (8) and possibly in hormonal-dependent cancer (9). There is a general agreement that estrogen effects on the thymus are mediated by specific receptors localized in epithelial thymic cells (10, 11). These observations offer new insight into the understanding of the possible mechanisms involved in the control of neuroendocrine immune functions.

Since it is now well established that the T-lymphocyte-derived growth factor IL-2, together with the expression of its receptor, plays a pivotal role in regulating the mitogen response to PHA (12), we studied the possibility of oxytocin modulating the response of peripheral blood mononuclear cells (PBMCs) to PHA, its ability to change both the expression of CD25 chain and the lymphocyte activation marker CD95. Furthermore, we studied both the inhibition of

PBMC blastic response to PHA induced by estradiol benzoate ($E_2B$) in the presence and absence of exogenous recombinant IL-2 and the IL-2 receptor (IL-2R) (CD25 chain) expression on the same lymphocytes. Finally, we assessed the possible capacity of oxytocin to reverse the inhibition of PBMC blastic response induced by $E_2B$.

## Patients and Methods

*Patients*. Fifteen healthy women were studied with a mean age of 33.8 (range 26-39) years, none having had previous pregnancies, all in the early follicular phase of the cycle, all with normal values of circulating estrogens (serum containing less than 75 pg/ml of estradiol). The protocol was approved by the Institutional Ethics Committee. Informed consent was obtained from all participants, according to the World Medical Association Declaration of Helsinki.

*Peripheral blood mononuclear cell separation*. Mononuclear cells were separated by Ficoll-Hypaque density gradient from freshly drawn heparinized peripheral blood and were washed three times in Hank's solution (Sigma-Aldrich, St. Louis, MO, USA). The cells were then kept in hormone-free RPMI-1640 medium (Sigma-Aldrich) at the required density and immediately cultured as described below.

*Peripheral blood mononuclear cell proliferative response assessed by flow cytometric cell cycle analysis*. The proliferative response of PBMCs to PHA and PHA plus oxytocin was evaluated as follows. Utilizing flat bottomed microtiter plates of 96 microwells (Falcon, Oxnard, CA, USA), $1.5\times10^5$ cells/well were cultured in triplicate in 200 μl of RPMI-1640 supplemented with 10% of autologous serum, 2 nmol glutamine (Flow Laboratories, Irvine, UK), 10 μg gentamicin (GIBCO, Invitrogen Ltd, Paisley, UK) and 1 μg of PHA (PHA-M; Boehringer, Ingelheim, Germany) or 1 μg of PHA plus $1\times10^{-10}$ M, $1\times10^{-11}$ M or $1\times10^{-12}$ M of oxytocin (Sigma Aldrich). To test whether the proliferative response was dependent on oxytocin binding to oxytocin receptor (OTR), and related activity, an antagonist of OTR, namely $d(CH_2)_5[Tyr(Me)^{2,Tyr4}$, $Tyr\text{-}NH_2]$ OVT (16-17) at $1\times10^{-9}$ M (13) was added to PBMC cultures stimulated with PHA plus oxytocin at the different concentrations given above. The oxytocin antagonist was gift from Dr Maurice Manning (Toledo, OH, USA). Mitogen responses to 1 μg PHA were also assayed in the absence and in the presence of 4, 20, 40 and 80 pg/0.1 ml estradiol benzoate ($E_2B$), with or without 20 IU of rIL2 (Boehringer) as described above. Cultures were incubated at 37˚C in a 5% $CO_2$ atmosphere for 72 hours. Each experiment was carried out in duplicate.

*Flow cytometric cell cycle analysis*. For flow cytometric cell-cycle analysis, $1\times10^6$ cells from each well of the plate were centrifuged at 300×g for 10 min, resuspended in 500 μl phosphate-buffered saline (PBS; lacking $Ca^{2+}$, $Mg^{2+}$ salt; Sigma-Aldrich), fixed in ethanol and stored at 2-8˚C until DNA staining. Before DNA analysis, cells were centrifuged (300×g, 10 min), washed twice in PBS and stained with 2 ml of PBS containing 50 μg/ml of propidium iodide (PI; Sigma-Aldrich) and 1 mg/ml RNAse (Roche Diagnostics, Milan, Italy) for 1 hour at 2-8˚C. DNA content was measured using a FACScan flow cytometer (Becton-Dickinson, Mountain View, CA, USA). The fluorescence of PI-stained nuclei, excited at 488 nm with 200 mW of laser power, was measured through a 620±40 nm band pass filter 20. List mode files were analyzed using CellQuest™ (Becton-Dickinson). Histograms of cell number *versus* linear integrated red fluorescence (proportional to DNA content) were recorded for a minimum of 10,000 nuclei (excluding internal standard) at flow rates no greater than 30 to 50 events per second. Isotonic saline solution (Facsflow; Becton-Dickinson) was used as sheath fluid. Cell cycle analysis of the DNA histograms of integrated red fluorescence was performed with ModFit™ (Becton-Dickinson). The software package includes algorithms for estimating multicut debris and aggregates: subtraction of debris and aggregates was performed in all samples prior to cell cycle analysis. The fluorescence was proportional to DNA content plus a normally distributed measurement error with a coefficient of variation (CV) calculated at $G_1$ DNA content and linearly decreasing to 0.85×CV at $G_2$ DNA content. A continuous distribution of DNA in the S-phase was assumed, the density being given as an exponential function of a second-degree polynomial defined between the $G_1$ and $G_2$+M means (14).

*Detection by flow cytometry of membrane bound CD25 and CD95 on PHA-stimulated PBMCs*. In the present study, cells at a density of $1.5\times10^6$/ml were incubated with PHA (10 μg/ml) in the presence of oxytocin at concentrations of $1\times10^{-10}$, $1\times10^{-11}$ and $1\times10^{-12}$ M. The incubation was performed for 72 h at 37˚C in a 5% $CO_2$ atmosphere. Subsequently the cells were washed twice in Hank's solution and resuspended at a density of $2\times10^6$/ml in RPMI-1640 solution. One hundred μl of this cell suspension were treated with 5 μl of fluorescein isothiocyanate (FITC)-conjugated monoclonal anti-CD25 antibody and 5 μl of phycoerythrin (PE)-conjugated monoclonal anti-CD95 antibody (both Becton Dickinson). Cells were incubated for 15 minutes at 4˚C, washed once with PBS and then resuspended in 400 μl of PBS. Thereafter, the CD25 and CD95 expressions were analyzed by flow cytometry (FACScan; BD Biosciences, Milan, Italy), and analyzed using CellQuest software (BD Biosciences). Considering that the proliferative response to mitogen, such as PHA, is associated with a characteristic enlargement of lymphocytes reflecting blastic transformation, changes in the morphology of mitogen-induced activation of PBMCs were monitored by flow cytometric analysis of cellular forward-scatter and side-scatter: according to size by forward angle and 90˚ light scattering properties, PBMCs were classified as total, blastic and nonblastic (6). CD25 expression was also analyzed on PHA-stimulated PBMCs in the presence of $E_2B$ (4, 20, 40, 80 pg/0.1 ml).

*Proliferative response and CD25 expression of PHA-stimulated PBMCs*. To verify whether oxytocin was able to reverse the inhibition of PBMC blastic response and/or CD25 expression induced by $E_2B$, the above experiments were repeated in the presence of $E_2B$ at the highest concentration plus oxytocin at the most effective dose.

*Statistical analysis*. All results are reported as the mean±standard deviation (M±SD). Comparison between values was performed by Student's *t*-test for paired data and ANOVA followed by Bonferroni correction. The cut-off for significance was a *p*-value of 0.01.

Table I. *Cytofluorimetric cell cycle analysis of PHA-stimulated PBMCs after 72 h of culture plus oxytocin with and without oxytocin antagonist.*

| Treatment | Phase (% cells) | | |
|---|---|---|---|
| | $G_0/G_1$ | S | $G_2/M$ |
| Unstimulated | 95.6±2.7 | 1.8±2.0 | 2.6±1.4 |
| PHA | 79.0±2.9[a*] | 15.8±3.6[a*] | 5.2±1.3[a*] |
| PHA+oxy$^{-10}$M | 63.8±11.0[b*§] | 29.3±6.8[b*§] | 6.9±4.2[*] |
| PHA+oxy$^{-11}$M | 63.0±7.8[b*§] | 29.6±5.0[b*§] | 7.4±3.4[b*] |
| PHA+oxy$^{-12}$M | 62.9±6.5[b*§] | 28.8±3.9[b*§] | 8.3±4.4[b*] |
| PHA+oxy antagonist$^{-9}$M | | | |
| + oxy$^{-10}$M | 75.9±16.1[c*] | 14.0±12.4[c*] | 9.9±3.5 |
| + oxy$^{-11}$M | 73.9±14.6[c*] | 15.1±11.8[c*] | 11.0±2.7[c] |
| + oxy$^{-12}$M | 73.3±12.9[c*] | 15.6±10.0[c*] | 11.1±2.9[c] |

PHA, Phytohemagglutinin; oxy, oxytocin. Student's *t*-test for paired data: [a]$p<0.001$ *versus* unstimulated cells, [b]$p<0.001$ *versus* PHA-stimulated cells, [c]$p<0.001$ *versus* oxytocin-stimulated cells); ANOVA test followed by Bonferroni's correction: [*]$p<0.001$ *versus* unstimulated cells, [§]$p<0.001$ *versus* PHA-stimulated cells.

## Results

*Proliferative response of PBMCs to PHA and PHA plus oxytocin with and without oxytocin antagonist*. PHA induced a significant proliferative response of PBMCs, similar to that we previously obtained (6). The addition of oxytocin ($1\times10^{-10}$ M, $1\times10^{-11}$ M, $1\times10^{-12}$ M) to the culture medium of PHA-stimulated PBMCs significantly increased the percentage of PBMCs that progressed into the S-phase of the cell cycle (Table I). The highest increase was observed when oxytocin was added at a concentration of $1\times10^{-10}$ M. In the presence of the oxytocin antagonist, the immunomodulating effects of oxytocin were no longer evident. In fact, the addition to cell culture of oxytocin antagonist at $1\times10^{-9}$ M significantly reduced the cell cycle progression of PHA-stimulated PBMC into the S-phase in comparison to cells treated with oxytocin alone (Figure 1).

*CD25 and CD95 expression on PHA-stimulated PBMCs*. A significant increase of CD25, CD95 and CD25/CD95 expression was observed both in blastic lymphocytes and in total lymphocytes in the presence of PHA in comparison to unstimulated cells and cells treated with PHA plus oxytocin in comparison to those treated with PHA alone. The addition of oxytocin antagonist at $1\times10^{-9}$ M significantly reduced the percentage of cells expressing both the CD25 and CD95 surface markers (Table II, Figure 2). Furthermore, oxytocin alone did not induce a significant change of CD25 and CD95 expression in total, blastic and nonblastic lymphocytes (data not reported).

The percentage of PHA-stimulated PBMCs expressing CD25 was significantly reduced by the presence of increasing concentration of $E_2B$ in the culture medium, for total, nonblastic and blastic lymphocytes (Table III).

Table II. *Cytofluorimetric analysis of PBMCs expressing CD25 and CD95 after 72 h of culture with PHA plus oxytocin with and without oxytocin antagonist.*

| | % PBMC | | |
|---|---|---|---|
| | Total | Nonblastic | Blastic |
| CD25$^+$ | | | |
| Unstimulated | 11.3±2.9 | 6.4±4.1 | 6.6±2.2 |
| PHA | 51.9±3.5[a*] | 24.0±2.0[a] | 27.0±8.5[a*] |
| PHA+oxy$^{-10}$M | 66.7±19.8[b*§] | 20.8±2.5[*] | 46.7±15.7[b*§] |
| PHA+oxy$^{-11}$M | 66.3±17.7[b*§] | 19.1±0.7[*] | 44.1±14.1[b*§] |
| PHA+oxy$^{-12}$M | 64.7±15.0[b*§] | 19.1±4.9[*] | 43.3±10.9[b*§] |
| PHA+oxy antagonist$^{-9}$M | | | |
| + oxy$^{-10}$M | 49.9±5.6[c*] | 20.9±8.3* | 26.2±2.4[c*] |
| + oxy$^{-11}$M | 51.4±11.9[c*] | 18.0±5.2[*] | 25.9±6.7[c*] |
| + oxy$^{-12}$M | 44.0±3.2[c*] | 17.9±5.0[*] | 25.8±7.3[c*] |
| CD95$^+$ | | | |
| Unstimulated | 24.9±3.2 | 13.8±4.9 | 11.5±2.5 |
| PHA | 51.1±13.7[a*] | 12.9±4.3 | 39.2±11.8[a*] |
| PHA+oxy$^{-10}$M | 69.3±12.3[b*§] | 8.2±1.9[*] | 57.0±3.2[b*§] |
| PHA+oxy$^{-11}$M | 67.0±15.6[b*§] | 9.8±2.7[*] | 55.1±4.2[b*§] |
| PHA+oxy$^{-12}$M | 59.9±9.7[b*§] | 8.0±3.6[*] | 49.0±7.9[*§] |
| PHA+oxy antagonist$^{-9}$M | | | |
| + oxy$^{-10}$M | 51.5±9.1[c*] | 14.4±13.9 | 36.5±8[c*] |
| + oxy$^{-11}$M | 48.9±10.7[c*] | 10.4±8.5 | 33.4±11.3[c*] |
| + oxy$^{-12}$M | 40.1±10.6[c*] | 13.9±7.6 | 39.5±6.2[c*] |
| CD95$^+$/CD25$^+$ | | | |
| Unstimulated | 7.4±3.5 | 3.2±0.9 | 4.8±2.6 |
| PHA | 43.8±16.5[a*] | 8.6±3.3[*] | 34.0±14.5[a*] |
| PHA+ oxy$^{-10}$M | 61.5±15.7[b*§] | 6.0±1.4[*] | 53.4±13.5[b*§] |
| PHA+ oxy$^{-11}$M | 58.6±7.7[b*§] | 7.2±1.9[*] | 50.4±17[b*§] |
| PHA+ oxy$^{-12}$M | 50.8±8.4[*§] | 5.2±0.9[*] | 45.6±19.2[*§] |
| PHA+oxy antagonist$^{-9}$M | | | |
| + oxy$^{-10}$M | 45.4±8.2[c*] | 9.8±8.5[*] | 34.6±0.8[c*] |
| + oxy$^{-11}$M | 43.7±8.7[c*] | 6.6±5.3[*] | 34.4±8.0[c*] |
| + oxy$^{-12}$M | 37.7±4.5[c*] | 6.9±5.1[*] | 27.1±6.4[c*] |

PBMCs, Peripheral blood mononuclear cells; PHA, phytohemagglutinin; oxy, oxytocin. Student's *t*-test for paired data: [a]$p<0.01$ *versus* unstimulated cells, [b]$p<0.01$ *versus* PHA-stimulated cells, [c]$p<0.01$ *versus* oxytocin-stimulated cells; ANOVA test followed by Bonferroni's correction: [*]$p<0.01$ *versus* unstimulated cells, [§]$p<0.01$ *versus* PHA-stimulated cells.

On PHA-activated PBMC, $E_2B$ at a dose of 80 pg/0.1 ml induced a marked decrease of CD25 expression both on blastic lymphocytes and on total lymphocytes This effect was not observed when oxytocin was added at $10^{-10}$ M (Table III).

*Proliferative response of PBMCs to PHA plus $E_2B$ with and without rIL-2*. For PHA-stimulated PBMC, $E_2B$ at the doses of 40 and 80 pg/0.1 ml induced a marked decrease in the percentage of cells progressing into the S-phase of the cell cycle, by 35% to 48%. The largest decrease was

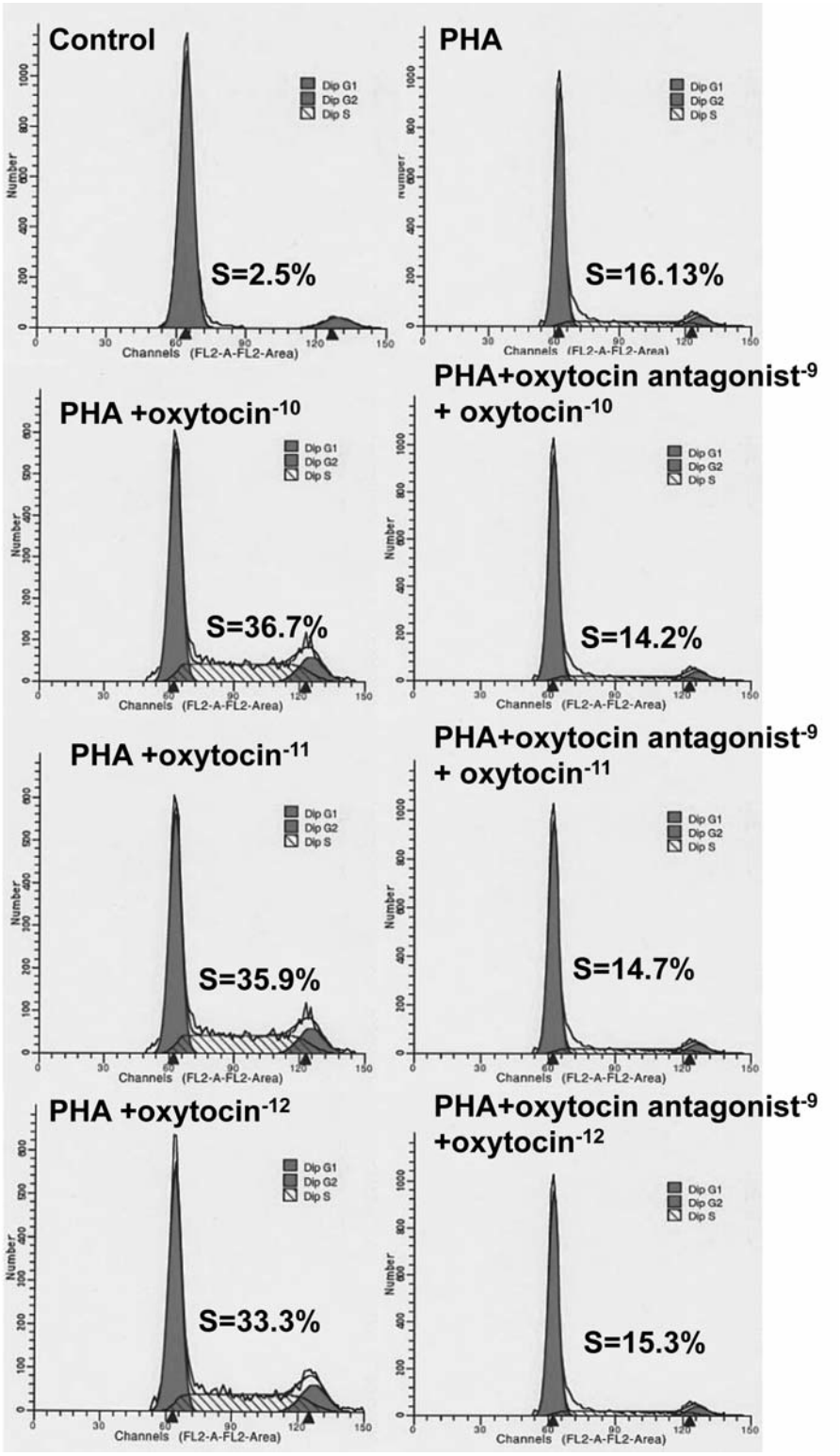


Figure 1. *Proliferative response of peripheral blood mononuclear cell (PBMCs) to phytohemagglutinin (PHA) and PHA plus oxytocin with and without oxytocin antagonist. The percentage of PBMCs in the S phase of the cell cycle after 72 hours of incubation with/without PHA, PHA plus oxytocin at $1\times10^{-10}$ M, $1\times10^{-11}$ M, $1\times10^{-12}$ M) with or without oxytocin antagonist at $1\times10^{-9}$ M are shown. The figure is representative of 15 independent experiments carried out in duplicate. The first peak represents the $G_0/G_1$ phase, the second peak the $G_2$+M-phase and the area between the two peaks corresponds to the S-phase.*

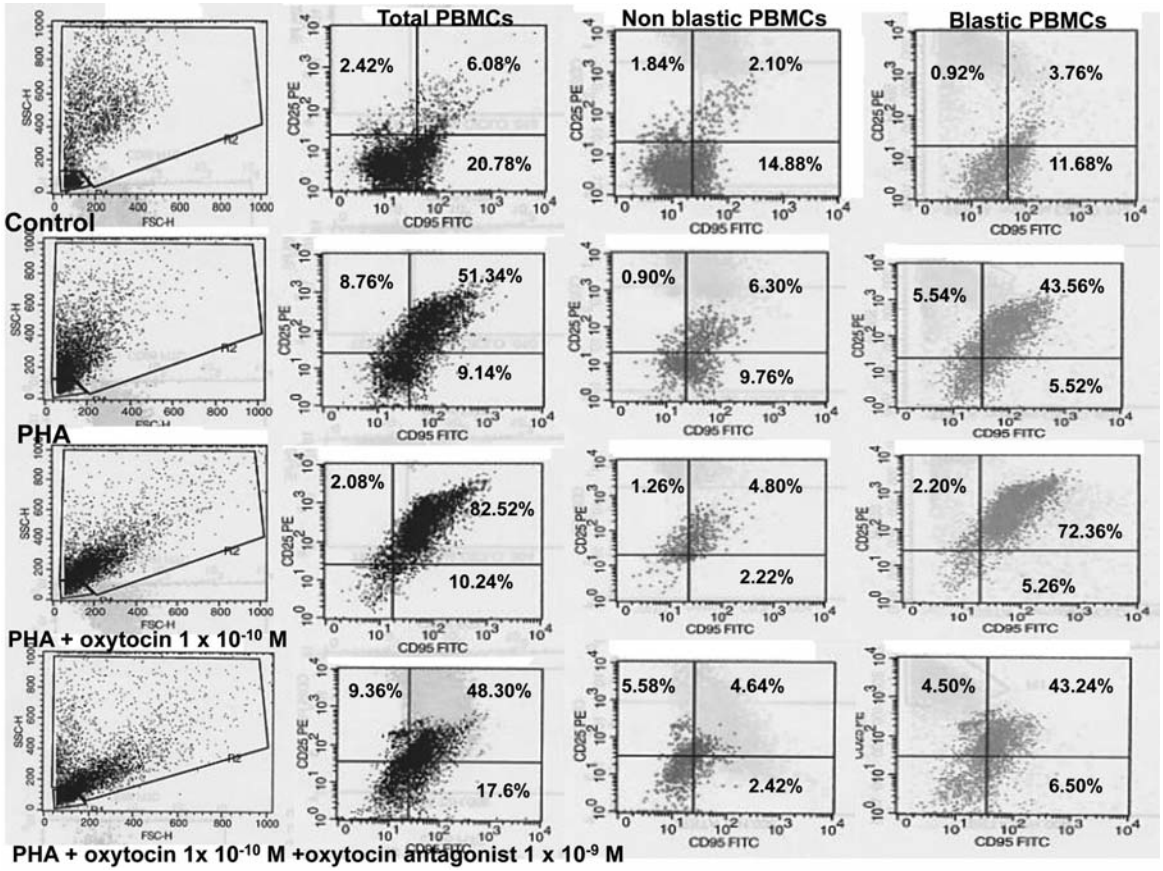


Figure 2. *Membrane-bound IL-2 receptor p55 chain (CD25) and surface activation marker CD95 expression on phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMCs) in the presence and in absence of oxytocin with and without oxytocin antagonist. The percentages of PBMCs expressing CD25 (PE-conjugated) and CD95 (FITC-conjugated) membrane-bound receptors in cultures in the presence of PHA, PHA plus oxytocin at $1\times10^{-10}$ M with and without oxytocin antagonist at $1\times10^{-9}$ M are reported. According to size by forward scatter (FSC, y axis) and 90° light side scattering (SSC, x axis) properties, PBMCs were classified as total, blastic and nonblastic. The figure is representative of 15 independent experiments carried out in duplicate.*

evidenced at $E_2B$ concentrations of 80 pg/0.1 ml. The addition of rIL-2 to the culture medium of PHA-stimulated PBMCs counteracted but did not completely reverse the inhibition induced by $E_2B$, which in this case ranged from 11% to 30% (Table IV).

The addition of oxytocin at a concentration of $1\times10^{-10}$ M to cell culture was able to reverse the inhibition of PHA-induced PBMC blastic response observed in the presence of the highest dose of $E_2B$ (80 pg/0.1 ml) (Table IV).

## Discussion

Extremely important in our study is the evidence that the neurohypophyseal peptide oxytocin is able to increase the PBMC blastic response to PHA as well as the expression of both the IL-2R (CD25 chain) and activation marker CD95 on the same lymphocytes. The present results also confirm and extend our previous findings showing that estrogens inhibit both the proliferative response to PHA and the expression of CD25 on PHA-stimulated PBMCs (6, 7, 15). Furthermore, with the aim of clarifying the immunoneuroendocrine connectivity and the paradigm of the oestrogen-hypothalamus/pituitary axis, we have shown that the inhibitory effect of oestrogen on PHA-activated PBMC proliferation was prevented by low concentrations of oxytocin, although this peptide by itself is devoid of mitogenic activity in non PHA-stimulated PBMCs. In this regard, it is important to consider that the reduced proliferative response of lymphocytes to mitogens, such as PHA, induced by estrogens should be considered as an index of more complex functional alterations. Indeed, these mitogens induce a number of phenomena *in vitro* similar to those that follow antigenic recognition *in vivo*. The secretion of macrophage cytokines, the production of IL-2 by $CD4^+$ lymphocytes and IL-2R expression on lymphocyte

Table III. *Cytofluorimetric analysis of PBMCs expressing CD25 after 72 h of culture with PHA and PHA+$E_2B$, with and without oxytocin.*

| | % PBMCs | | |
|---|---|---|---|
| Treatment | Total | Nonblastic | Blastic |
| PHA | 51.9±3.5 | 24.0±2.0 | 27.0±8.5 |
| PHA+$E_2B$ 4 pg/0.1 ml | 52.6±2.0 | 24.1±1.9 | 26.0±5.4 |
| PHA+$E_2B$ 20 pg/0.1 ml | 50.0±1.6 | 23.0±1.0 | 25.4±4.3 |
| PHA+$E_2B$ 40 pg/0.1 ml | 43.4±2.5[a*] | 20.0±1.0[a*] | 23.1±2.4[*] |
| PHA+$E_2B$ 80 pg/0.1 ml | 39.0±9.1[a*] | 17.6±4.6[a*] | 22.0±2.8[a*] |
| PHA+$E_2B$ 80 pg/0.1 ml +oxy$^{-10}$M | 46.0±12.4[b] | 18.9±3.2[*] | 26.0±10.1 |

PBMCs, Peripheral blood mononuclear cells; PHA, phytohemagglutinin; $E_2B$, estradiol benzoate; oxy, oxytocin. Student's *t*-test for paired data: [a]$p<0.01$ *versus* PHA, [b]$p<0.01$ *versus* PHA plus $E_2B$; ANOVA test followed by Bonferroni's correction: [*]$p<0.01$ *versus* PHA.

Table IV. *Cytofluorimetric cell cycle analysis of PHA-stimulated PBMCs after 72 h of culture in the presence of estradiol benzoate with and without exogenous recombinant IL-2 20 IU or oxytocin.*

| | % PBMCs | | |
|---|---|---|---|
| Treatment | $G_0/G_1$ | S | $G_2/M$ |
| Unstimulated | 95.6±2.7 | 1.8±2.0 | 2.6±1.4 |
| PHA | 79.0±2.9[a*] | 15.8±3.6[a*] | 5.2±1.3[a*] |
| PHA +$E_2B$ 4 pg/0.1 ml | 80.2±3.9[*] | 14.4±3.2[*] | 5.0±1.1[*] |
| PHA +$E_2B$ 20 pg/0.1 ml | 81.2±2.3[*] | 13.4±2.5[*] | 4.7±2.3[*] |
| PHA +$E_2B$ 40 pg/0.1 ml | 84.4±2.3b[*§] | 10.1±4.3[b*§] | 5.0±2.1[*] |
| PHA +$E_2B$ 80 pg/0.1 ml | 86.3±3.4b[*§] | 8.2±3.2[b*§] | 5.5±1.5[*] |
| PHA+$E_2B$ 4 pg/0.1 ml+rIL-2 | 80.2±6.5[*] | 15.2±5.1[*] | 4.7±2.7[*] |
| PHA+$E_2B$ 20 pg/0.1 ml+rIL-2 | 82.3±7.6[*] | 14.4±4.6[*] | 4.2±2.2[*] |
| PHA+$E_2B$ 40 pg/0.1 ml+rIL-2 | 83.1±5.3[c*] | 12.2±3.7[*] | 4.6±1.7[*] |
| PHA+$E_2B$ 80 pg/0.1 ml+rIL-2 | 84.6±4.2[c*] | 11.2±3.9[c*] | 5.4±1.9[*] |
| PHA+$E_2B$ 80 pg/0.1 ml +oxy$^{-10}$M | 71.7±13.9[c*] | 20.0±12.5[c*] | 8.3±3.2[c*] |

PBMCs, Peripheral blood mononuclear cells; PHA, phytohemagglutinin; $E_2B$, estradiol benzoate; rIL-2, recombinant interleukin-2. Student's *t*-test for paired data: [a]$p<0.01$ *versus* unstimulated PBMCs, [b]$p<0.01$ *versus* PHA, [c]$p<0.01$ *versus* PHA plus $E_2B$; ANOVA test followed by Bonferroni's correction: [*]$p<0.01$ *versus* unstimulated cells, [§]$p<0.01$ *versus* PHA-stimulated cells.

membrane are the defining moments of these events. The degree of the lymphocyte blastic response thus depends on the quantity of cytokines produced, mainly of IL-2, the number of IL-2R expressed and the physiological interaction of this cytokine with its receptor (6). It is thus likely that the estrogen effect on PHA-activated PBMCs may be due to an inhibition of IL-2R CD25 chain, following PHA stimulation, as the blastic response of PBMCs to PHA is directly related not only to the production of endogenous IL-2, but also, and equally, to the expression of IL-2R (7, 12, 15, 16). Accordingly, in the present study, the addition of rIL-2 to the culture medium of PHA-stimulated PBMCs attenuated, although not completely, the inhibition induced by $E_2B$. However, there is no doubt that the activity of oxytocin is selective and is due to interaction with its specific receptor present on PBMCs. In fact, the addition to the culture medium of an oxytocin antagonist reverses the immunomodulating activity of oxytocin. Indeed, it has been shown that oxytocin receptors are expressed by both thymic immature T-cells and by mature cytotoxic $CD8^+$ lymphocytes (17).

The capacity of oxytocin to increase the expression of CD25 and CD95 on PHA-stimulated PBMCs may in turn enable this peptide to improve the lymphocyte blastic response to PHA (15, 16, 18). Accordingly, the prevention of the inhibitory effect of estrogens on PHA-induced PBMC proliferation by oxytocin, led us to speculate that this neurohypophyseal peptide, well known mainly for its hormonal role in delivery and lactation, might be involved in the modulation of PBMC response prccisely through the modulation of the percentage of $CD25^+$ activated cells induced by polyclonal mitogen PHA.

In this regard, it is important to remember that the human thymus produces appreciable amounts of oxytocin, mainly in the so-called thymic nurse cells (1) and that estrogens not only affect mature PBMCs directly by acting on specific receptors (19), but also act at an earlier stage of T lymphocyte maturation by modifying, as a consequence of their hormonal action, the thymus environment, in which the maturation and differentiation of these takes place (9). Indeed, estrogens reduce the ability of thymic epithelial cells to synthesize thymic hormones (5), which play an essential physiological role in the maturation of thymic lymphocytes (20). Thus, while the presence of oxytocin in the thymus suggests an active role for this neuropeptide in the proliferation, differentiation and selection of T lymphocyte lineage (17), its ability to potentiate the blastic response to PHA and to prevent estrogen-induced inhibition seems to indicate that this hormone can also play a role in modulating the immune response of mature T lymphocytes also by modifying their function in the periphery (21). Our results lead us to hypothesize that the oxytocin effect is due to an increase in the expression of IL-2 R (CD25 chain), and to a possible increase in endogenous IL-2 synthesis. The exact mechanism(s) by which oxytocin modifies the response of PBMCs to PHA, in the absence and presence of estrogens, is unknown at present but this activity may involve the induction of a second messenger such as cyclic GMP (22). Indeed, both PHA as well as oxytocin elicited a functional $[Ca^{2+}]$ response in T lymphocytes through the activation of cyclic nucleotides, thus initiating a variety of

intracellular events, including cell proliferation and protein synthesis (23, 24). Although further studies are needed to clarify the mechanism(s) by which oxytocin influences response of PBMCs to PHA and, more generally, the T-cell immune function, and consequently whether this effect plays a role in the recovery of a normal maternal immune function at the end of pregnancy, the present results further support the hypothesis that neuropeptides may act as a link in the network between the central nervous, the immune and the endocrine systems (25).

## Acknowledgements

This work was supported by the Associazione Sarda per la Ricerca in Oncologia Ginecologica ONLUS.

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*Received August 20, 2009*
*Revised January 5, 2010*
*Accepted January 5, 2010*