Acta Physiologica Hungarica, Volume 90 (3), pp. 207–215 (2003)

Endorphin content of white blood cells and peritoneal cells in neonatally benzpyrene treated adult rats

G **Csaba**[1], P **Kovács**[1], Éva **Pállinger**[2]

[1] Department of Genetics, Cell and Immunobiology, Semmelweis University and
[2] Molecular Immunological Research Group of the Hungarian Academy of Sciences, Budapest, Hungary

Received: February 10, 2003
Accepted: April 15, 2003

White blood cells of rats (lymphocytes, monocytes, macrophages, granulocytes and mast cells) contain β-endorphin. Two months after a single neonatal benzpyrene treatment (imprinting) there is an elevated level of immunoreactive endorphin in the blood and peritoneal cells of female animals and blood cells of males. The endorphin content decreased in the peritoneal cells of males. In the blood, the granulocytes of female, and the lymphocytes of male rats contained the highest amount of endorphin. In the peritoneal fluid also the granulocytes of females contained the highest amount of endorphin, in contrast to males, where the endorphin content of cells decreased and the lowest level of it was present in the lymphocytes.

The experiments justify that benzpyrene treatment can durably influence endorphin levels of white blood cells and gives new data to the already known lifelong health destroying effects of perinatal benzpyrene exposition (alterations of hormone receptor binding capacity and sexual behavior).

Keywords: benzpyrene, endorphin, mast cell, granulocyte, monocyte, lymphocyte, hormonal imprinting

Endogeneous opioids (primarily β-endorphin) are produced and released not only by brain cells, but also by the cells of immune system (lymphocytes and monocytes), first of all during inflammation (3, 21). In our earlier experiments (13) the presence of β-endorphin in granulocytes was also demonstrated. These opioids are able to reduce pain caused by the inflammatory process and they also reduce inflammation, while increasing the chemotaxis of immune cells (4, 15).

Neonatal interactions can influence different physiological processes for life. In this time hormonal imprinting is taking place, when the hormone and its developing

Correspondence should be addressed to
G. Csaba MD, PhD, DSc
Department of Genetics, Cell and Immunobiology
Semmelweis University
H-1445 Budapest, POB. 370, Hungary
E-mail: csagyor@dgci.sote.hu

0231–424X/2003/$ 20.00

target receptor first meet (6–8). Imprinting is needed for the normal maturation of receptor – signal transduction – hormone system (9). However, the excess of hormone-like molecules (e.g. members of the same hormone family, synthetic hormone analogues, drugs or environmental pollutants with hormone-like structure) can cause faulty imprinting (6–8) with life-long consequences, which are manifested at gene, receptorial, morphological, biochemical, even sexual behavioral levels (1, 2, 11, 17, 19, 22). In addition, imprinting with a hormone can enhance the lifelong production of the same hormone (12). Endorphins are also targets of these neonatal interactions. In earlier experiments, administration of sexual steroids to newborn rats elevated the immunoreactive β-endorphin level of pituitary gland (16) and reduced it in the medial preoptic area as well, as in the mediobasal hypothalamus (14) in adult rats. Since the environmental pollutant benzpyrene has a steroid-like structure and in previous experiments it provoked faulty imprinting of steroid receptors with extended effects (10, 11), it was reasonable to study the effect of neonatal benzpyrene treatment on the endorphin content of white blood cells and cells of the peritoneal fluid, in adult animals.

Materials and Methods

Newborn male and female animals of our closed (Charles River originated) Wistar breed, benzpyrene (Sigma, USA) was given in a dose of 50 μg/animal (solved in sunflower oil), subcutaneously. Controls received the vehicle. When the animals were 2 months old, isotonic Na citrate was injected (during ether anesthesia) into the peritoneal cavity which was regained after 30 sec. After that, blood was obtained by cardiac puncture into isotonic Na citrate solution. Six control and 6 benzpyrene treated animals were studied in the experiments.

Before labeling, erythrolysis was done (by using Becton Dickinson FACS Lysing Solution). The lysed red blood cell remnants were removed by washings in PBS and the cells were fixed in 4% paraformaldehyde solution. After that, the cells were permeabilized with 0.1% saponin. The β-endorphin content of permeabilized cells were detected with antibody to endorphin (produced in rabbit, Sigma, USA) used as primary antibody, and FITC-labelled anti-rabbit FITC- IgG (Sigma), as secondary antibody. For controlling the specificity, autofluorescence of the cells and aspecificity of the secondary antibodies were detected. The measurement was done in a FACSCalibur flow cytometer (Becton Dickinson, San Jose, USA), using 10,000 cells for each measurement. For the measurement and analysis CellQuest 3.1 program was used.

During the evaluation, cell populations had been separated on the basis of size and granulation and defined by “gating”. In the identical cell populations the serotonin content inside the cells had been compared. The numerical comparison of detected values was done by the comparison of percentual changes of geometric mean channel values to the control groups.

After the flow cytometric analysis the cells were subjected to confocal microscopic analysis in a BioRad MRC 1024 confocal laser scanning microscope, equipped with krypton-argon mixed gas-laser as a light source, at an excitation wavelength of 480 nm line. All experiments were repeated twice.

Results and Discussion

White blood cells produce, store and secrete β-endorphin. However it is important to know that the total endorphin amount of immune cells is not more, than one-third of the amount produced by the pituitary gland (21). Nevertheless, this smaller amount of endorphin could play an important role in immune homeostasis (3, 21).

In earlier experiments – studying the serotonin content of adult's immune cells after neonatal serotonin treatment – gender differences were observed: the serotonin content of cells increased, however only in the female animals (13). A similar situation was observed in the case of neonatal endorphin treatment mentioned above, when in the pituitary gland the endorphin content increased after neonatal steroid treatment only in females (16). This was the reason why we studied the endorphin content separately in the two sexes.

In female rats the white blood cells contained endorphin and in the neonatally benzpyrene treated animals its level was elevated in each cell type, however the level was the highest in granulocytes (Fig. 1). In the same cells of the peritoneal fluid there was also an elevated level of endorphin and in mast cells – which also contain endorphin – a higher level could be observed, too (Fig. 2). Confocal microscopic studies support the data of the flow cytometric analysis, demonstrating the presence and distribution of the opioid in the cells as well, as the more intense fluorescence of the cells in the treated animals (Fig. 3).

In the blood of male rats the endorphin level increased in each cell type (Fig. 4), with the highest level in the lymphocytes. However, in the cells of peritoneal fluid a decrease of endorphin content was observed (Fig. 5), with the lowest level in the lymphocytes. Confocal microscopy supported the flow cytometric data (Fig. 6).

Using Student's *"t"* method, the difference between the control and neonatally benzpyrene treated animals was not significant, because of the very high deviation between the individual controls as well as the individual treated rats (which was similar to each other). This could be the consequence of stress effects, which increase the concentration of the opioid in these cells several fold (21). This means that the endorphin content of white blood cells is moving in a very broad range (5, 20). However, the mean of this level was consistently higher in the benzpyrene treated female animals and higher or lower – depending on the type (blood or peritoneal) of the cells – in male animals, and the values were different from the appropriate controls (in the direction mentioned above) in each case.

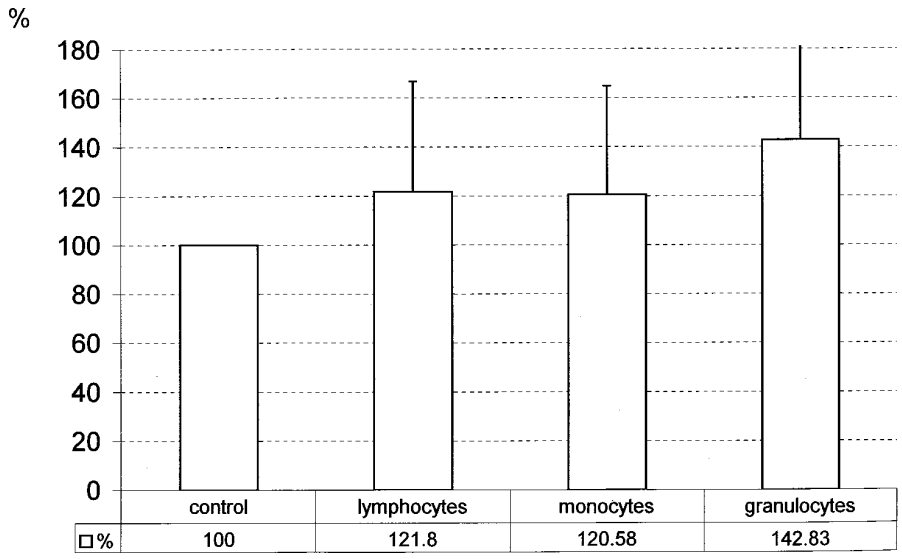

Fig. 1. Endorphin content of neonatally benzpyrene treated adult female rat's white blood cells, related to the control as 100%

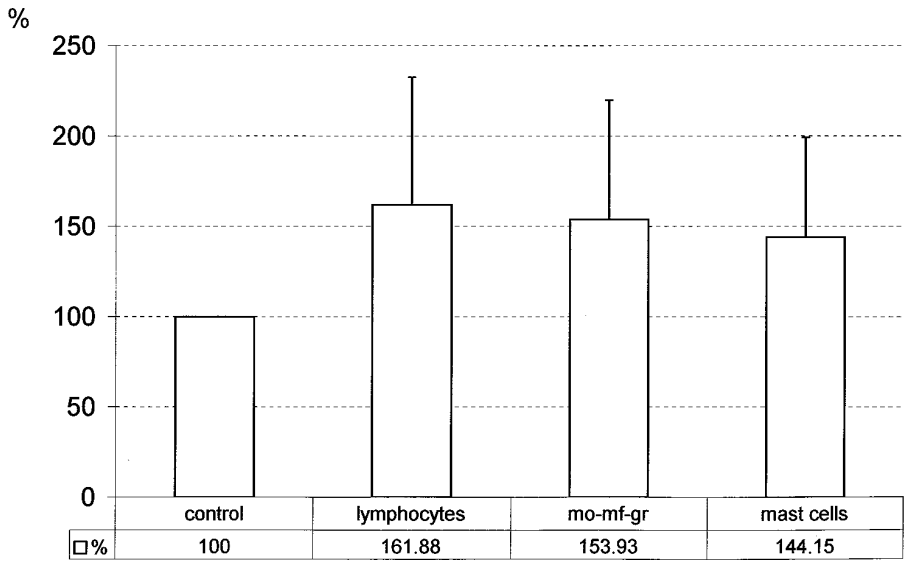

Fig. 2. Endorphin content in the peritoneal cells of neonatally benzpyrene treated adult female rats, related to the control as 100%

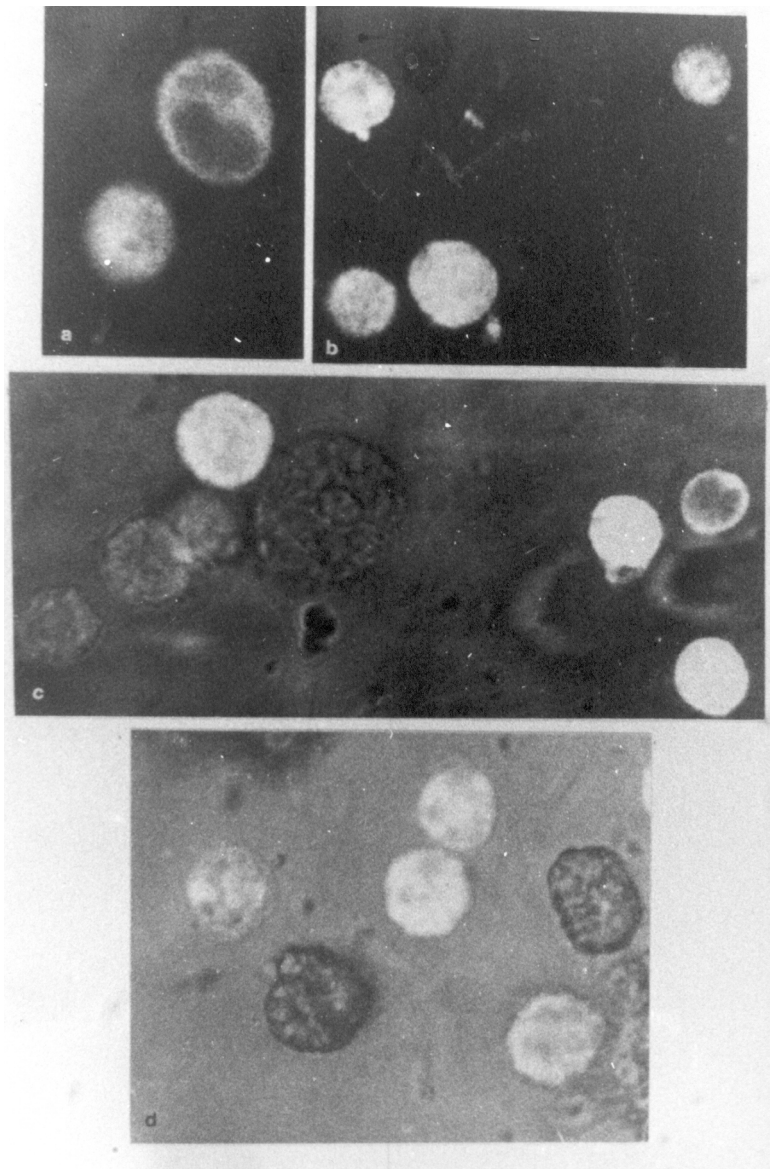

Fig. 3. Confocal microscopic pictures of control (a) and neonatally benzpyrene treated (b) adult female rat's white blood cells and combined fluorescent and transmission photograph of control (c) and neonatally benzpyrene treated (d) female rat's peritoneal cells. These latters demonstrate that not all of the cell types show fluorescence

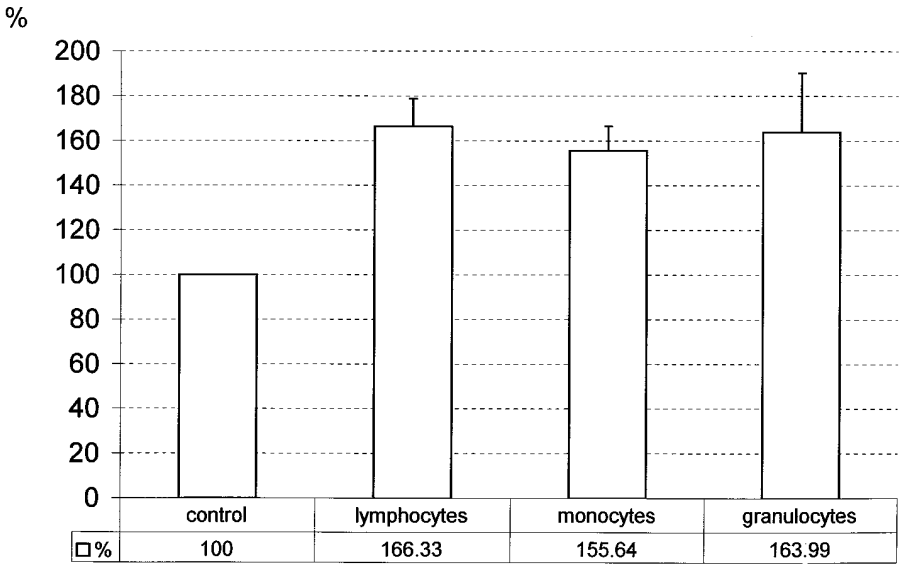

Fig. 4. Endorphin content of neonatally benzpyrene treated adult male rat's leukocytes, related to the control as 100%

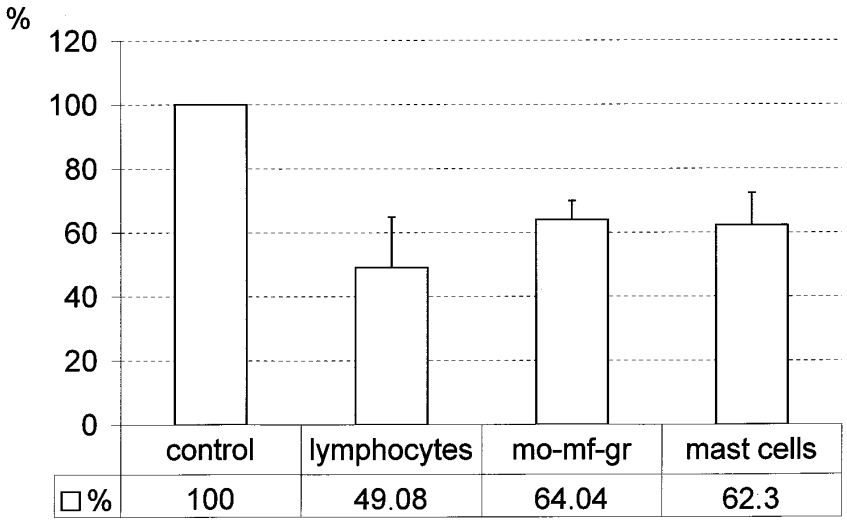

Fig. 5. Endorphin content in peritoneal cells of neonatally benzpyrene treated adult male rats, related to the control as 100%

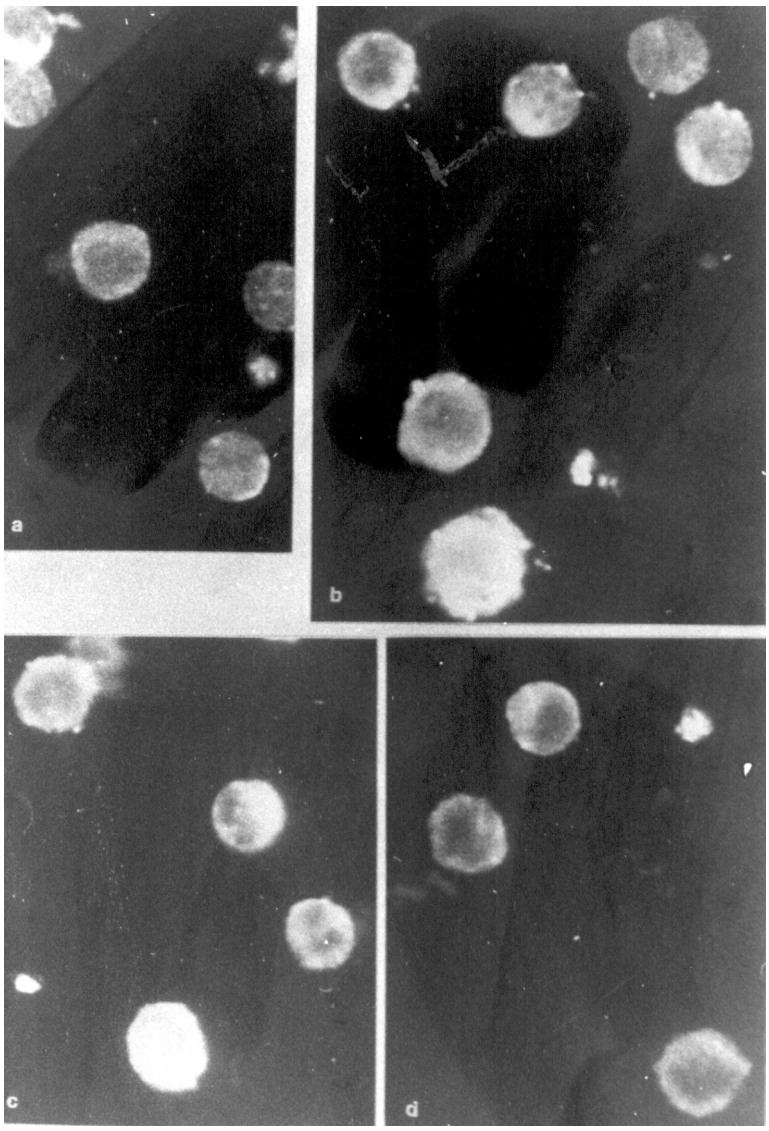

Fig. 6. Confocal microscopic pictures of control (a) and neonatally benzpyrene treated (b) leukocytes, and control (c) as well as neonatally treated (d) peritoneal cells of adult male rats. An elevation of endorphin fluorescence in blood leukocytes and a decrease in peritoneal cells of treated animals, can be observed

The results call attention to the gender differences in the endorphin content of immune cells and at the same time to the differences between the identical cells in different milieu. Though we do not know the reason of the differences between the

endorphin content of blood and peritoneal cells, considering the immuno-regulatory function of endorphin, this would be important in case of inflammation or local immune reaction.

Benzpyrene is a pollutant which is practically always present in urban environment. In earlier experiments the life-long harmful effect of neonatal treatment with this molecule to steroid hormone receptor binding capacity and sexual behavior was demonstrated (7, 10, 11). The present experiments point to the prolonged effect on the endorphin content of white blood cells. On the basis of the experiments it cannot be exactly decided whether these alterations are useful or harmful, only the effect can be registered. However, knowing that endorphins have a role in immune homeostasis by depressing immunological functions (21), and modulating immunocompetence by stimulating T cell proliferation (18), the altered level in immune cells after neonatal benzpyrene exposition could point to an important harmful effect.

Acknowledgements

This work was supported by the National Research Fund (OTKA T-042513), Hungary. The authors thank Ms. Katy Kallay and Ms. Angela Kozák for the expert technical assistance.

REFERENCES

1. Bern HA, Gorski RA, Kawashima S: Long term effects of prenatal hormone administration. Science 181, 189–190 (1973)
2. Bern HA, Jones LA, Mori T, Young PN: Exposure of neonatal mice to steroids: long term effects on the mammary gland and other reproductive structures. J. Steroid Biochem. 6, 673–676 (1975)
3. Blalock JE: β-endorphin in immune cells. Immunol. Today 19, 191–192 (1998)
4. Cabot PJ, Carter L, Gaiddon C, Zhang Q, Schafer M, Loeffler JP, Stein C: Immune cell-derived beta endorphin. Production, release, and control of inflammatory pain in rats. J. Clin. Invest. 100, 142–148 (1997)
5. Conti F, Pittoni V, Sacerdote P, Priori R, Meroni PL, Valesini G: Decreased immunoreactive beta-endorphin in mononuclear leucocytes from patients with chronic fatigue syndrome. Clin. Exp. Rheumatol. 16, 729–732 (1998)
6. Csaba G: Phylogeny and ontogeny of hormone receptors: the selection theory of receptor formation and hormonal imprinting. Biol. Rev. 55, 47–63 (1980)
7. Csaba G: Phylogeny and ontogeny of chemical signaling: Origin and development of hormone receptors. Int. Rev. Cytol. 155, 1–48 (1994)
8. Csaba G: Hormonal imprinting: its role during the evolution and development of hormones and receptors. Cell Biol. Int. 24, 407–414 (2000)
9. Csaba G, Nagy SU: Influence of the neonatal suppression of TSH production (neonatal hyperthyroidism) on response to TSH in adulthood. J. Endocrin. Invest. 8, 557–559 (1985)
10. Csaba G, Inczefi-Gonda Á, Szeberényi Sz: Lasting impact of a single benzpyrene treatment in pre-natal and growing age on the thymic glucocorticoid receptors of rats. Gen. Pharmac. 22, 815–818 (1991)
11. Csaba G, Karabélyos Cs, Dalló J: Fetal and neonatal action of polycyclic hydrocarbon (benzpyrene) or a synthetic steroid hormone (allylestrenol) as reflected by the sexual behaviour of adult rats. J. Developm. Physiol. 19, 67–70 (1993)

12. Csaba G, Kovács P: Effect of neonatal interleukin-6 (IL-6) treatment (hormonal imprinting) on the IL-6 content and localization of the peritoneal, blood and thymic cells of adult rats. A confocal microscopic analysis. Cell Biol. Int. 25, 1179–1182 (2001)
13. Csaba G, Kovács P, Pállinger E: β-endorphin in granulocytes. Cell. Biol. Int. 26, 741–744 (2002)
14. Diez-Guerra FJ, Bicknell RJ, Mansfield S, Emson PC, Dyer RG: Effect of neonatal testosterone upon opioid receptors and the content of beta-endorphin, neuropeptide Y and neurotensin in the medial preoptic and the mediobasal hypothalamic areas of the rat brain. Brain Res. 424, 225–230 (1987)
15. Epps van DE, Saland L: Beta-endorphin and met-enkephalin stimulate human peripheral blood mononuclear cell chemotaxis. J. Immunol. 132, 3046–3053 (1984)
16. Forman LJ, Estilow S: Administration of gonadal steroids to neonatal rats affects beta-endorphin levels in the adult. Life Sci. 40, 687–695 (1987)
17. Gray-Nelson K, Sakay Y, Eitzman B, Steel T, McLachlan JA: Exposure to diethylstilbestrol during a critical developmental period of the mouse reproductive tract leads to persistent induction of two estrogen-regulated genes. Cell Growth Diff. 5, 115–119 (1994)
18. Hemmick LM, Bidlack JM: Beta-endorphin stimulates rat T lymphocyte proliferation. J. Neuroimmunol. 29, 239–248 (1990)
19. Iguchi T: Cellular effect of early exposure to sex hormones and antihormones. Int. Rev. Cytol. 139, 1–57 (1992)
20. Marchini M, Manfredi B, Tozzi L, Sacerdote P, Panerai A, Fedele L: Mitogen-induced lymphocyte proliferation and peripheral blood mononuclear cell beta endorphin concentrations in primary dismenorrhoea. Human Repr. 10, 815–817 (1995)
21. Panerai AE, Sacerdote P: β-endorphin in the immune system: a role at last. Immunol. Today 18, 317–319 (1997)
22. Tchernitchin A, Tchernitchin N: Imprinting of paths of heterodifferentiation by prenatal or neonatal exposure to hormones, pharmaceuticals, pollutants and other agents and conditions. Med. Sci. Res. 20, 391–397 (1992)