NPY and VIP neuropeptides endogenous factors in the induction of preterm delivery labour

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Summary: The Authors wanted to verify whether neuropeptides like VIP and NPY have a role in regulating the production of PGE2 by the amniotic membrane, by the use of a continuous cell line of amniotic derivation as a model of study. The study was carried out in the laboratories of the 4th Division of the 2nd Institute of the Maternity Home of the Umberto I General Hospital in Rome.

Key words: Neuropeptides; Prostaglandines; Preterm delivery.

INTRODUCTION

According to the present O.M.S. definition, the expulsion of the foetus after the 180th day of delivery, up to the 259th day included, as from the beginning of last menstruation (1), is called preterm delivery. Frequency rises along with the increase in the age of pregnancy, varying between 6% and 15% of all deliveries and it is still the most important cause of perinatal mortality and morbidity (2).

Notarthstanding the importance of this clinical problem, the mechanism which causes preterm delivery labour in women is still unknown. In recent years, special attention has been given to two substances, the NPY (neuropeptide Y) and VIP vasoactive intestinal peptide) neuropeptides. They are produced by many cells of the central nervous system (3). The physiological role of the NPY during pregnancy is related to the role of catecolamines; as a matter of fact the NPY is found, together with the norepinephrine, in the nervous terminations and with the epinephrine in the suprarenal medulla. The levels of the catecolamines rise within the amniotic liquid in the same way as the NPY. Similar observations have been made on the vasoactive intestinal polypeptide (4).

The object of this work was to evaluate whether neuropeptides as NPY and VIP have a role in regulating the production of PGE2 by the amniotic membrane. With this aim in view we have used the pattern of human amniocites in culture.

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MATERIALS AND METHODS

a) *Materials*

WISH cells (ATCC, 25-CCL, Rockville, Maryland).

DMEM (Dulbecco's modified minimum essential medium); FCS (fetal calf serum; Flow Laboratories).

Penicillina-streptomicina (Flow Laboratories).

L-glutammina; VIP Neuropeptide Y (kindly granted by Prof. F. Petraglia, Modena).

Plates with 96 pits 6 mm in diameter each (Linbro, Flow Laboratories).

b) *Cell culture*

Tests were carried out using WISH cells, a cell line deriving amniotic tissues which had already been characterized.

The cells, spread into plates and concentrated by 20,000 per pit, were grown with Dulbecco's modified minimum essential medium adding 10% fetal calf serum, penicilline (50 UI/ml), L glutamine (2 mM).

The cells were grown in plates having 96 pits 6 mm in diameter each until reaching the confluence that is achieved after about 3 days of incubation. After 3 days the cells were conditioned for 24 hours with 1% serum, and thereafter washed with culture medium without serum; the cultures were then put to incubate with VIP and Neuropeptide Y at 37 degrees centigrade for 24 hours.

c) *Evaluation of protein content*

At the end of the incubation period the culture terrain was taken away from each pit. The pits were washed with terrain without serum and 200 microliters of NaOH 0.2 N were added in each one, putting them in incubation for 12 hours at 37 degrees centigrade.

At the end of such period the solution 0.2 N of NaOH was removed from each pit and an equal volume of HCl 0.2 N was added.

Protein content was measured by using Bio Rad method described by Bradford in 1976.

d) *Evaluation of PGE2 production*

The quantity of PGE2 produced was measured directly in the culture medium by means of a radioimmunologic essay [PGE2 (125 I) RIA kit, NEN-Dupont].

The results are expressed as production of PGE2 per microgram of protein.

All tests were doubled and the data drawn are given in tables 1 and 2 which show the average ± standard deviations.

RESULTS

The production of prostaglandines by WISH cells in culture is remarkably modified by treatment with NPY and VIP without serum (Tables 1 and 2).

The remarkable increase in the production of PGE2 induced by NPY seems to be dependant on the dose (Table 1), the opposite of what occurs for VIP (Table 2).

Table 1. — *NPY results on the production of PGE2 (pg/nanog. prot) by WISH cells in culture.*

pm	500	50
NPY	184.13 ± 149.9	92.3 ± 6.2
checking	1.63 ± 1.3	

These values show the averages of the replicates ± standard deviations (5 pits per observation point).

Table 2. — *VIP results on the production of PGE2 (pg/nanog. prot.) by WISH cells in culture.*

pm	500	50
VIP	59.9 ± 28.8	64.23 ± 42.3
checking	1.63 ± 1.3	

These values show the averages of the replicates ± standard deviations.

CONCLUSIONS

It is believed that preterm delivery can be caused by the abnormal stimulation or inhibition of a single system which contributes, along with many other factors, in the induction of delivery ([5]). The excitation or inhibition of this single sytem can be produced by many "inputs", both exogenous and endogenous ([6]). Our study was focussed on the latter, by using neuropeptides NPY and VIP for this purpose, showing that they bring about an increase in the production of PGE2 by an amniaotic line in culture.

This may evidence that these neuropeptides have a role in the biochemical events occuring in labour; as a matter of fact, prostaglandines increase the intensity of uterine contractions and when administered to women, they induce both labour in term pregnancy and abortion during the first and second quarter ([7]). Concentration of NPY is very high especially during pregnancy and stress in labour makes its levels rise even further ([8]). On the whole, these observations may evidence that these neuropeptides have a role in the biochemical events occuring in labour.

We consider it necessary that a deeper understanding of the roles of VIP and NPY be achieved in order to work out new therapeutical strategies for the treatment of preterm delivery.

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