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Biochimica et Biophysica Acta 1266 (1995) 186–190

BBA Biochimica et Biophysica Acta

Electrophysiological effects of progesterone on hepatocytes

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Received 5 August 1994; accepted 1 December 1994

Abstract

The addition of progesterone (1–100 μmol/l) to the extracellular fluid bathing rat hepatocytes led to a rapid and fully reversible depolarization of the cell membrane. The progesterone-induced depolarization was paralleled by a decrease of potassium selectivity and an increase of cell membrane resistance and was abolished in the presence of the potassium channel blocker barium. Accordingly, in whole cell recordings, progesterone led to a decrease of the cell membrane conductance. 17 α-Hydroxyprogesterone and β-estradiol were less effective by a factor of 10, whereas cholesterol, corticosterone and hydrocortisone did not significantly alter the potential difference across the cell membrane. In conclusion, acute administration of progesterone depolarized rat hepatocytes by decreasing the potassium conductance of the cell membrane.

Keywords: Hepatocyte; Progesterone; Cell membrane potential; K^+-Conductance

1. Introduction

Intrahepatic cholestasis is a relatively common complication of pregnancy [1–3]. The mechanism underlying this disorder, however, remains elusive. Progesterone has been reported to inhibit the Na^+/K^+ ATPase in cardiac cells [4] and it has been shown that progesterone depolarizes Madin Darby canine kidney (MDCK) cells by inhibition of the K^+-conductance [5]. Both effects, if they were to occur in the liver, would be expected to compromise bile secretion. The present study has been performed in order to elucidate the effect of progesterone on the cell membrane potential of liver cells and to investigate a possible effect of the hormone on the ion conductances of the cell membrane.

2. Materials and methods

Experiments were performed on rat hepatocytes prepared according to Hansen's technique [6].

The cells were grown (1–3 days) in culture dishes in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 100 g/l fetal calf serum (FCS), 100 U/ml penicillin, and 100 μg/ml streptomycin at 37° C, 5% CO_2 and 95% humidified air.

For the determination of cell membrane potential, dishes with incompletely confluent cell layers were mounted into a perfusion chamber (volume: 0.1 ml; perfusion rate: 10 ml/min). The isotonic solution was composed of (in mmol/l): 115.0 NaCl, 5.0 KCl, 1.0 $MgCl_2$, 1.3 $CaCl_2$, 2.0 NaH_2PO_4, 18.0 $NaHCO_3$ and 5.0 glucose. Bicarbonate-containing solutions were equilibrated with 5% carbon dioxide and 95% air (pH 7.4).

All experiments applying conventional electrophysiology were performed at 37° C, the whole cell recordings were performed at room temperature. Measurements of the potential difference across the cell membrane (PD) were made using conventional microelectrodes (tip diameter < 0.5 μm, input resistance 100–200 MΩ, tip potential < 5 mV), back-filled with 1 mol/l KCl, connected to a high input impedance electrometer (FD223 WPI, New Haven, CT). Measurements were made versus an Ag/AgCl electrode connected to the bath via a 3 mol/l KCl-agar bridge. Impalements were made under an inverted phase-contrast microscope (Axiovert 135, Zeiss, Oberkochen, FRG), using a piezostepper (PM 20 N, Frankenberger, Germering, FRG) mounted on a Leitz micromanipulator (Leitz, Wetzlar, FRG). To determine the input resistance before, during and after impalement square wave pulses up to 50 pA were injected by a stimulator (Grass Instruments,

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SSDI 0167-4889(94)00236-3

Quincy, MA, USA) and the voltage deflection was used to calculate the input resistance. Recordings were accepted only under conditions in which the input resistance was similar before and after the impalement. The input resistance is either the electrode resistance or, during intracellular recording, the sum of electrode resistance and apparent cell membrane resistance. The latter was determined from the difference between input resistance during impalement and input resistance before and after the impalement. The apparent potassium transference number (t_K), a measure of the potassium selectivity of the cell membrane, can be calculated according to [7,8]:

$$t_K = \Delta PD_K / \Delta EMF_K$$

ΔPD_K is the voltage deflection and ΔEMF_K the alteration of potassium equilibrium potential if extracellular potassium concentration is altered from 5.0 to 20 mmol/l. A decrease of t_K results in a respective decrease of ΔPD_K. The potassium conductance (G_K) can be expressed as $G_K = t_K/R_m$ (R_m ... cell membrane resistance). Thus, a simultaneous decrease of ΔPD_K and increase of R_m reflect a decrease of G_K. Determination of both, t_K and R_m may be subject to errors: The calculation of t_K may be biased by a change of the conductance or EMF for other ions. The error may be minimized, if the PD at 20 mmol/l K^+ is close to the EMF for the ions other than K^+ [7,8]. Determination of R_m may be biased by any reversible change of microelectrode resistance, e.g., by a change of fluid resistivity around the electrode tip. The resistance measurements with conventional microelectrodes were, however, confirmed by the whole cell recordings.

Whole cell patch clamp experiments were performed with single cells at room temperature using conventional patch electrodes with a resistance of 4–6 MΩ when the pipette was filled with the internal solution (in mmol/l): 5.0 NaCl, 115 KCl, 1.0 $MgCl_2$, 0.5 $CaCl_2$, 2.0 NaH_2PO_4, 5.0 glucose, 10 Hepes and 1.0 EGTA. The solution was adjusted with KOH to pH 7.15. The bath solution (chamber volume 0.5 ml, perfusion rate 5 ml/min) was composed of (in mmol/l): 115 NaCl, 5.0 KCl, 1.0 $MgCl_2$, 1.3

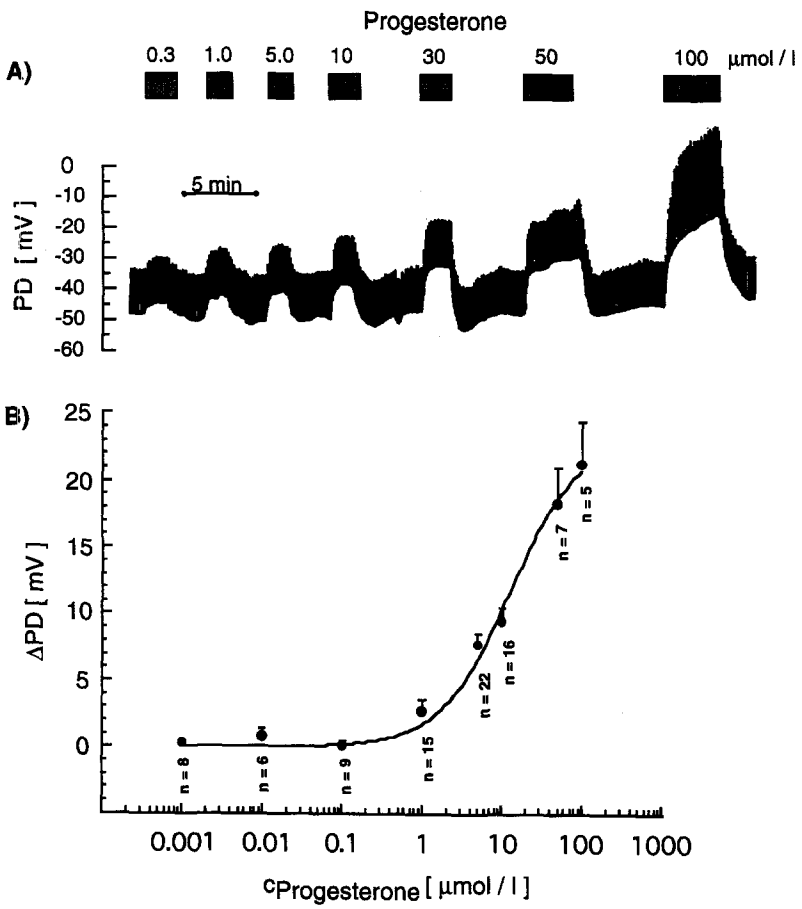

Fig. 1. A: Original tracing showing effects of progesterone (0.3, 1.0, 5.0, 10, 30, 50, 100 μmol/l) on potential difference across cell membrane (PD) of a rat hepatocyte. The PD was determined with conventional microelectrodes. The voltage deflections were due to the injection of 50 pA via the electrode and reflect the sum of electrode and cell membrane resistance. Progesterone was added to the perfusate (symbolized by bars) at the concentrations indicated. B: Dose–response curve of the depolarizing effect (ΔPD) of progesterone. Arithmetic means ± SEM, n = number of cells studied. The line reflects a least square fit of Michaelis Menten kinetics with a Hill coefficient of 1.

$CaCl_2$, 2.0 NaH_2PO_4, 5.0 glucose and 18 HEPES. The solution was adjusted to pH 7.4 with NaOH.

The whole cell current recordings were performed using a patch clamp amplifier (EPC9, HEKA, Lambrecht, Germany). The currents were measured at holding potentials in the range of 0 to −20 mV and during 200-ms pulse potentials ranging in 10-mV steps from −50 mV to +30 mV at 15-s intervals. After normalizing the current values to the cell surface assuming a unit capacity of 1 $\mu F/cm^2$ linear current voltage relations were calculated (cell membrane capacities were in the range of 33 to 76 pF).

Where indicated, progesterone and/or $BaCl_2$ were added at the concentrations as specified. Progesterone was kept in a stock solution at a concentration of 1 mmol/l in ethanol and pipetted to the bath solution shortly before the experiment. In experiments with 10 mmol/l $BaCl_2$ $NaHCO_3$ and NaH_2PO_4 were replaced by Tris buffer (5 mM).

Applicable data are expressed as arithmetic means ± standard error of the mean (S.E.M.). Statistical analysis was made by paired or unpaired *t*-test, where applicable.

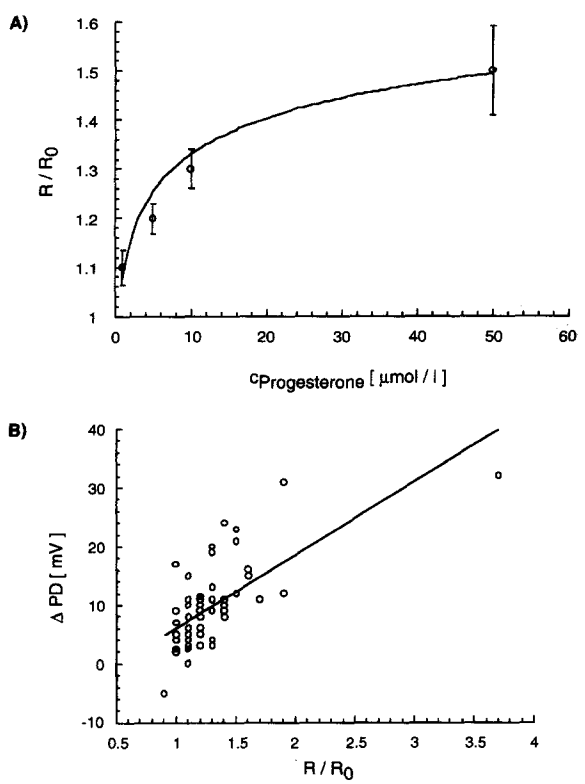

Fig. 2. A: Change of cell membrane resistance (R/R_0) as a function of progesterone concentration in the bath (R is the resistance during and R_0 the resistance before application of the hormone). The cell membrane resistance was determined from the alterations of voltage deflection due to current injection via the microelectrodes. The line reflects a least square fit of Michaelis Menten kinetics with a Hill coefficient of 1. B: Progesterone-induced change of cell membrane potential (ΔPD) as a function of progesterone-induced change of cell membrane resistance (R/R_0, where R is the resistance during and R_0 the resistance before application of the hormone).

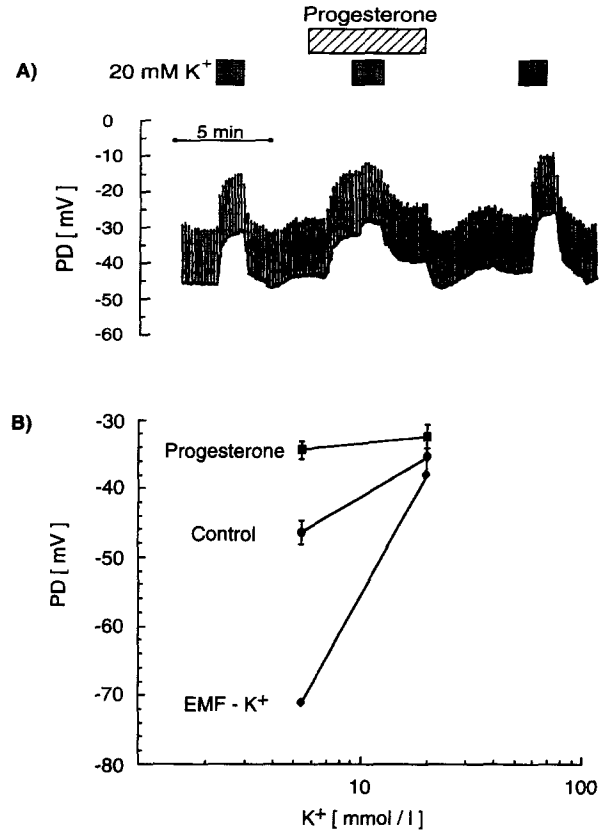

Fig. 3. A: Original tracing illustrating the effect of increasing extracellular K^+ concentration from 5.0 to 20.0 mmol/l on the potential difference across the cell membrane both in the absence and presence of progesterone (5 µmol/l). The PD was determined with conventional microelectrodes. The voltage deflections were due to the injection of 50 pA via the electrode and reflect the sum of electrode and cell membrane resistance. The bars symbolize the application of 20 mmol/l KCl and progesterone as indicated. B: Cell membrane potential (PD) as a function of extracellular potassium concentration in absence (control conditions) and presence of progesterone (5 µmol/l), $n = 7$; EMF-K^+ indicates the respective equilibrium potentials for potassium.

Statistically significant differences were assumed at $P < 0.05$.

3. Results

In the absence of progesterone the potential difference across the rat hepatocyte cell membrane was −44.7 ± 0.9 mV ($n = 46$). Progesterone (5 µmol/l) depolarized the cell membrane by 7.7 ± 0.9 mV ($n = 22$). The progesterone-induced depolarization was rapid and fully reversible. Fig. 1 depicts the dose–response curve of the progesterone-induced depolarization.

The depolarization following addition of progesterone (5 µmol/l) was paralleled by an increase of input resistance (by 15.7 ± 3%, $n = 21$).

As shown in Fig. 2, the progesterone-induced depolarization and increase of input resistance were correlated.

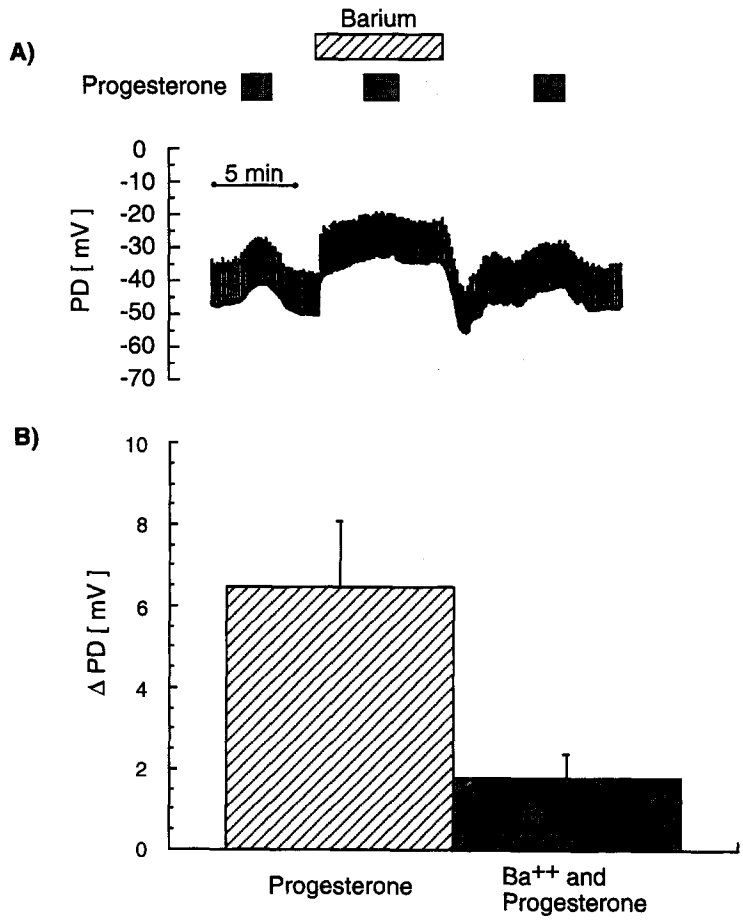

Fig. 4. A: Original tracing illustrating the effect of progesterone (5 μmol/l) on the potential-difference across the cell membrane both in the absence and presence of Ba^{++} (10 mmol/l). The PD was determined with conventional microelectrodes. The voltage deflections were due to the injection of 50 pA via the electrode and reflect the sum of electrode and cell membrane resistance. B: Depolarizing effect of progesterone (5 μmol/l) under control conditions (left column) and in the presence of barium (10 mmol/l, right column); $n = 4$.

When up to 10 μmol/l of other steroids such as cholesterol, hydrocortisone, corticosterone were applied, no significant alteration of PD was observed.

β-Estradiol (50 μmol/l) and 17α-hydroxy-progesterone (50 μmol/l) depolarized the cell membrane potential by 5.0 ± 0.9 mV ($n = 4$) and by 7.5 ± 1.2 mV ($n = 6$), respectively.

Rapid increase of the potassium concentration from 5.0 to 20 mmol/l depolarized the cell membrane potential by 12.1 ± 1.5 mV ($n = 7$) in the absence and by 3.0 ± 0.6 mV ($n = 9$) in the presence of progesterone (Fig. 3), resulting in a decrease in the apparent potassium transference number t_K by 78.7 ± 9.2% ($n = 9$). In view of the simultaneous increase of cell membrane resistance, the decrease of t_K reflects a reduction of the potassium conductance of the cell membrane.

Ba^{++} (10 mmol/l), a blocker of potassium channels, depolarized the cell membrane by 18.3 ± 2.3 mV ($n = 4$). In the presence of Ba^{++}, the depolarizing effect of progesterone (5 μmol/l) was reduced from 6.5 ± 1.6 mV ($n = 4$) to 1.8 ± 0.6 mV ($n = 4$) (Fig. 4).

In whole cell recordings (Fig. 5), the cell membrane conductance was 7.8 ± 1.3 nS ($n = 10$) in the absence of the hormone. Following addition of progesterone (10 μmol/l) the cell membrane conductance decreased by 24.1 ± 4.1% to 5.8 ± 1.0 nS ($n = 10$) and the potential at zero current was shifted from −25.8 ± 2.9 mV to −12.7 ± 2.6 mV ($n = 10$). The difference between zero current potential and cell membrane potential as recorded with conventional microelectrodes may result from the difference of temperature, bath and cytosolic ion composition.

4. Discussion

The electrophysiological effects of progesterone, i.e., the depolarization of the cell membrane, the increase of cell membrane resistance and the decrease of the K^+ selectivity of the cell membrane, all point to an inhibitory effect of progesterone on K^+ channels in the hepatocyte cell membrane. Furthermore, the depolarizing effect of progesterone is virtually abolished in the presence of the potassium channel blocker barium. If progesterone acted by stimulation of a depolarizing conductance, as for exam-

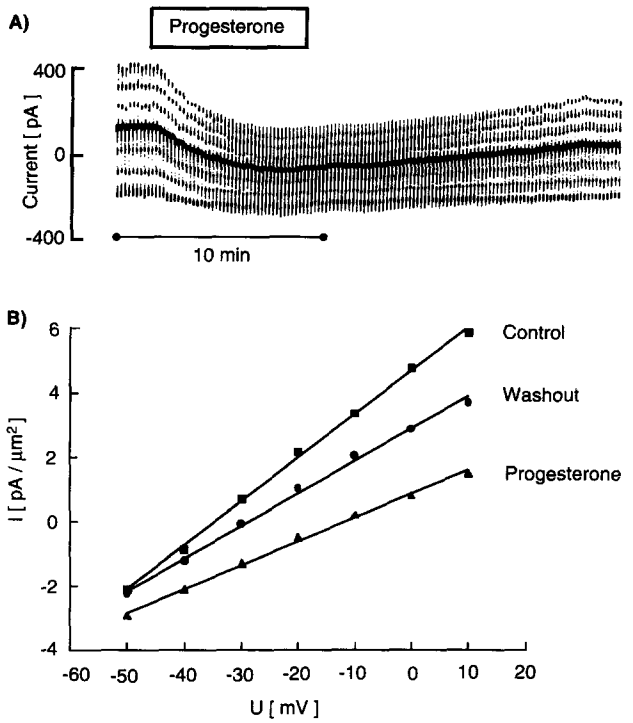

Fig. 5. A: Original tracing illustrating the effect of progesterone (10 μmol/l) in whole cell patch clamp studies. The currents were measured at a holding potential of −20 mV and during 200-ms test pulse potentials ranging in 10-mV steps from −50 mV to 10 mV at 15-s intervals. B: Current voltage relations obtained from the tracing in Fig. 5A. The currents were normalized to the cell surface assuming a unit capacity of 1 μF/cm^2 (cell membrane capacity 71 pF). The voltages were corrected for the voltage drop across the series resistance (12 MΩ) due to the pipette tip. Control, current voltage relationship before application of progesterone; Washout, current voltage relationship after withdrawal of progesterone.

ple a chloride-conductance, its effects should be augmented by an inhibition of potassium channels. The progesterone-induced reduction of cell membrane conductance together with the decrease of the reversal potential suggest the inhibition of a hyperpolarizing conductance. However, additional effects on other channels such as anion channels cannot be excluded.

The effect fully paralleled the effects of progesterone on the electrical properties of MDCK cells [5]. In those cells, a similar depolarization has been observed, paralleled by an increase of cell membrane resistance and a decrease of K^+ selectivity of the cell membrane.

The rapid onset and reversibility of the observed progesterone effect renders a genomic mechanism highly unlikely. Several non-genomic actions of progesterone have been observed in a great variety of cell types which seem to be mediated by different cell membrane receptors [9–13]. Furthermore, progesterone could reduce the potassium conductance by direct interference with potassium channels.

The concentrations required to elicit the progesterone-induced depolarization are high but still in the range of concentrations encountered during pregnancy [14–16]. It must be kept in mind that not progesterone as such but a cellular metabolite may be effective.

Whether the inhibition of the potassium conductance contributes to the impaired bile secretion as it is seen in intrahepatic cholestasis of pregnancy remains to be tested. In any case, the inhibitory effect of progesterone on K^+ channels in hepatocytes reveals an as yet unknown hepatic action of the hormone which may alter cellular function at pathophysiologically high concentrations of the hormone.

Acknowledgements

The skilled technical support of Irina Öttl, Vera Janle, Birgitta Noll and Christiane Arnold is gratefully acknowledged. This study was supported by the Deutsche Forschungsgemeinschaft, grant La 315/4-1.

References

[1] Rustgi, V.K. (1989) Med. Clin. North. Am. 73 (4), 1041–46.
[2] Reyes, H. and Simon, F.R. (1993) Semin. Liver Dis. 13 (3), 289–301.
[3] Anday, E.K. and Cohen, A. (1990) Ann. Clin. Lab. Sci. 20 (4), 233–238.
[4] Bose, D., Elliott, D., Kobayashi, T., Templeton, J.F., Kumar, V.P.S. and Labella, F.S. (1988) Br. J. Pharmacol. 93, 453–461.
[5] Steidl, M., Pinggera, G., Ritter, M. and Lang, F. (1991) Am. J. Physiol. 260 (5), E743–E750.
[6] Hansen, C. A., Mah, S. and Williamson, J. R. (1986) J. Biol. Chem. 261, 8100–8103.
[7] Lang, F., Oberleithner, H. and Giebisch, G. (1986) Am. J. Physiol. 251, F1063–F1072.
[8] Völkl, H. and Lang, F. (1988) Eur. J. Physiol. 411, 514–519.
[9] Fitz, J.G., Persico, M. and Scharschmidt, B.F. (1989) Am. J. Physiol. 256 (3 Pt 1), G 491-G 500.
[10] Sanchez-Bueno, A., Sancho, M.J. and Cobbold, P.H. (1991) Biochem. J. 280 (1), 273–276.
[11] Foresta, C., Rossato, M. and Di Virgilio, F. (1993) Biochem. J. 294 (1), 279–283.
[12] Valera, S., Ballivet, M. and Bertrand, D. (1992) Proc. Natl. Acad. Sci. USA 89 (20), 9949–9953.
[13] Ke, F.C. and Ramirez, V.D. (1990) J. Neurochem. 54 (2), 467–472.
[14] Paaby, P., Nielson, A. and Raffin K. (1989) Acta Endocrinol. 120, 636–643.
[15] Cunningham, D.S., Brodnik, R.M., Rayl, D.L., Brown, A.W. and Hansen, K.A. (1993) J. Reprod. Med. 38 (4), 301–305.
[16] Buyalos, R. P., Glassman, L. M., Rifka, S.M., Falk, R.J., Macarthy, P.O., Tyson, V.J. and DiMattina, M. (1992) J. Reprod. Med. 37 (3), 261–266.