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Thesis of Master's Degree

LOCALIZATION OF DOPAMINE AND DOPAMINE D2 RECEPTOR IN THE HUMAN PLACENTA, FETAL MEMBRANES AND UMBILICAL CORDS BY IMMUNOHISTOCHEMISTRY

FACULTY OF MEDICINE

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ADVISORS

Professor SONG CHANG HUN MD.,PhD.

Professor PARK CHANG SOO MD.,PhD.

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2004 November

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ABSTRACT

LOCALIZATION OF DOPAMINE AND DOPAMINE D2 RECEPTOR IN THE HUMAN PLACENTA, FETAL MEMBRANES AND UMBILICAL CORDS BY IMMUNOHISTOCHEMISTRY

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During human pregnancy, the placenta is the major site to feed the fetus, many hormones are synthesized and maintain high concentrations in the maternal circulation. It has been reported that the human placenta could produce many neuropeptides, growth factors, and cytokines. Dopamine is one of these hormones. Dopamine exerts its effects through interactions with dopamine receptors. Dopamine receptors have been divided into two types: D1 and D_2 receptor. Dopamine D1 or D_2 receptor has been proved their presence in the human placenta, in fetal membranes by many authors, but research about localization of the dopamine is very few.

The aim of this study was to determine the localization of Dopamine and Dopamine D_2receptors in the human placenta, fetal membranes and umbilical cords of normal pregnancy at term by immunohistochemistry analysis, using the immunohistochemical ABC methods with rabbit anti-dopamine polyclonal antibody AB122s and polyclonal antibody rabbit anti-dopamine D_2receptor AB1558 as primary antibody. Immunoreactive

was localized in the syncyotiotrophoblastic cells and trophoblastic cells and deciduas of menbrane. With one hundred fifty seven stains, the positive was 33.3% (14/42), 25% (5/20), 38.5% (3/8) for Dopamine , and 48% (24/26), 56% (14/25), 50% (6/12) for Dopamine D2 receptor in placenta, fetal membranes and umbilical cords.

According to our results, Dopamine and Dopamine D_2 receptors have a relationship with placenta. We propose the hypothesis that Dopamine is present in human placenta. This study is the first toshow the presence of Dopamine in human placenta, and Dopamine D_2 receptor have also an activity on the placenta. Dopamine and Dopamine D_2 receptor were not only showed in human placenta but also in the fetal membranes and umbilical cords.

It is speculated that placenta may produce Dopamine, Dopamine D_2receptor. May be they play a more important role in the human placenta development and pregnancy maintenance, and also take part in regulating fetal development.

Key words: Dopamine, Dopamine D_2 receptors, placenta, fetal membrane, umbilical cord, Immunohistochemistry.

I. INTRODUCTION:

Dopamine, Nor-epinephrine, and epinephrine belong to a class of neurotransmitters known as catecholamines which are structurally defined by a catechol ring and an amine side chain. Dopamine is catecholamine neurotransmitter found in neurons of both the central and peripheral nervous system. It is stored in vesicles in axon terminals and released when the neuron is depolarized and is synthesized primarily in the central nervous system (CNS), but limited production also occurs in the adrenal medulla. Dopamine interacts with specific membrane receptors to produce its effects. These effects are terminated by reuptake into the presynaptic neuron by a Dopamine transporter, or by metabolic inactivation by monoamine oxidase B (MAO-B) or catechol-omethytransferase (COMT) (1). Dopamine is also detectable in a few non-neuronal tissue, e.g. the pancreas and anterior pituitary gland (21). Dysfunction of dopaminergic systems is associated with a number of diseases. An imbalance between the dopaminergic andcholinergic systems cause the trouble of function in the body, when dopamine is excessive or Acetycholine (Ach) depleted, excessive movement or hyperkinetic state results (movement disorders). The converse, decreased dopamine or increase Ach, leads to bradykinesia, rigidity and sometimes tremor (Parkinson's disease). Distributionand physiology of Dopamine outside the brain are not well definite.

Dopamine receptors have been studied intensively due to their prominent role in modulating locomotor function, reproduction and emotional states. Agonists and antagonists of Dopamine receptors are used widely to treat disorders such as Parkinson's disease and schizophrenia, respectively. Moreover, center nervous system appear to mediate the behavioral effect of drug abuse like cocaine by including locomotor activities, stereotypies and reward seeking behavior.

Dopamine synthesized by tubero-infundibular neurons in the hypothalamus, is delivered to the anterior pituitary through a hypothalamo-hypophysial portal system and is a strong inhibitor of prolactin gene _expression. The inhibitory role of DA is mediated by Pit-1), it has been reported thay DA

inhibit PL secretion from human placenta (Vaillancourt 1993,1994,1997). This control of DA is mediated through a D2 receptor binding and inhibition of cAMP synthesis, just as in the pituitary lactotroph. It is presumed that DA regulated PL gene _expression through the mediation of Pit-1 transactivation (20).

Five different subtypes of dopamine receptor have been identifiedin the mammalian. These are divided into to major groups: D1- like types or D1 receptor subfamily and D2- like types or D2 receptor subfamily. D1 receptor subfamily included D1 and D5 receptor subtypes, D2 receptor subfamily included D2, D3 and D4 receptor subtypes (1).

In 1979, Kubota et al was described in the literature the presence of dopamine in human placenta endocrine function. After that, there were many authors wanted to understand more about the relationship between placenta and Dopamine, but all most these reports proved Dopamine receptor either D1 receptor or D2 receptor.

In the 1990s, Vaillancourt et al published some reports about dopamine D2 receptor in human trophoblastic cells as D2 dopamine agonists inhibit adenosine 3': 5' cyclic monophosphate (cAMP) production in human term trophoblastic cells (2), continuously they had a series of the research about Dopamine receptor in human placenta as: Inhibition of Angiotensin-stimulated inositol phosphate production by D2-dopamine receptor is calcium dependent in human trophoblastic cells (3), Interaction of D2-dopamine receptor with two pertussis toxin sensitive G proteins in human placenta, 1997; _Expression of human placental D2-dopamine receptor during normal and abnormal pregnancies (4), and _expression of human placenta Dopamine D2 receptor during normal and abnormal pregnancies (5). Other research of Yanagawa et al, in the same time, was presence of dopamine DA-1 receptor in human deciduas (19).

Recently, there are the articles related dopamine and placenta of Hyun Joon Kim et al with "The localization of dopamine D2 receptor mRNA in the human placenta and the anti-angiogenic effect of apmorphine in the chorioallantoic membrane". (8). And Elwan et al (12) in characterization of

dopamine D2 receptor gene _expression and binding sites in human placenta amniotic epithelial cells.

All of above research were often focused on Dopamine receptor D1, or D2 in the human placenta.

However, the localization of Dopamine in placenta was very few. Can placenta secrete Dopamine and Dopamine receptors? What are their functions in placenta? The source of dopamine in human placenta is still unknown

The aim of this study is to determine the localization of the Dopamine and compare with Dopamine D2 receptor in the human placenta, fetal membranes and umbilical cords by immunohistochemistry .Which cells produced them and what are their functionsin placenta and fetal development? And we suppose the hypothesis that placenta can secrete Dopamine and also Dopamine D2 receptor.

II. MATERIALS AND METHODS

1. Specimen studies

Placental tissues

One hundred fifty seven samples placentae, fetal membranes and umbilical cord at full term from normal tissue uncomplicated pregnancies were obtained include seventy specimens were stainedwith Dopamine, and eighty seven stained with Dopamine D2 receptor after delivering normal or cesarean section at term from the Chosun University hospital in Kwangju (Table1).

Each placenta piece was embedded in paraffin and 5μm section were prepared

Table 1: Distribution of Dopamine and Dopamine D2 receptor stain in human placenta, fetal membranes and umbilical cords.

	DA	DA D2 receptor	Total
Placentae	42	50	92
Fetal membranes	20	25	45
Umbilical cords	8	12	20
Total	70	87	157

2. Immunohistochemistry

Immunohistochemical procedure.

The paraffin sections were dewaxed.. Then, they were stained according to the immunohistochemical ABC methods. Tissue sections were incubated at room temperature for 24 hours in the primary antibody AB 122s Rabbit anti-Dopamine (1:100 dilution), and AB1558s Rabbit anti-Dopamine D2 receptor polyclonal antibody (1:250 dilution) from Chemicon International (Temecula, CA 92590, USA).

The secondary antibody, Biotin-labelled horse anti-rabbit IgG was incubated at room temperature for 10 minutes at room temperature and ABC complex was incubated. After rinsing in PBS the slides were incubated on streptavidin-HRP for 8 minutes and on 3,3- diaminobenzidine (DAB) for

10 minutes at room temperature, (Vectastain elite ABC kit, Vector Laboratories) .The section was counterstained with hematoxylin, air-dried and coverslipped.

3. Control procedure:

For control purpose, specimens of the same tissue were stained as described above, but either omitting the primaryantibodies or replacing them by antibody dilution buffer, nonimmune rabbit serum.

III. RESULTS

1. Localisation of Dopamin

Immunohistochemistry show Dopamine cells as dark brown, background as light yellow or unstained, with immunopositive cells being easily identified. All the syncytiotrophoblasts in villi of human placenta study had immunoreactive Dopamine in their cytoplasm . The cytotrophoblasts showed weak Dopamine immunoreaction.

The Dopamine immunoreactive material was seen in the cytoplasm of the capillary endothelium of placenta villi.

Fig 1, Fig.2, Fig3.: Immunoreactivity was showed in syncytiotrophoblast, trophoblast

Fig 4: Slide control immunonegative of Dopamine in placenta

Fig.1

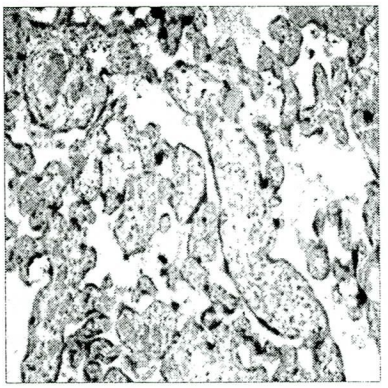


Fig2

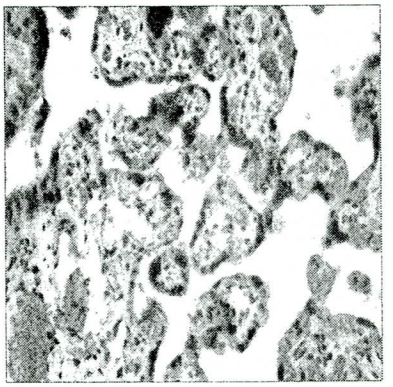


Fig.3

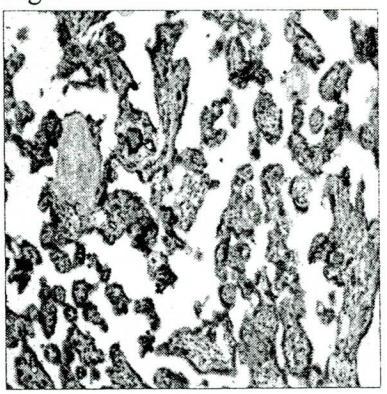
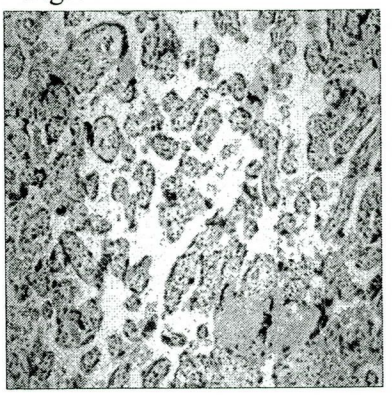


Fig.4



2. Localisation of Dopamin D2 receptor in placenta

Cells immunopositive for Dopamine D2 receptor had dark brown reaction product in the cytoplasm. The syncytiotrophoblast and trophoblast all showed DA D2 receptor immunoreactivity, with the reaction intensity being stronger in syncytiotrophoblast than in trophoblast. The capillary endothelium, fetal white blood cells in the capillary cavity of placenta villi showed immunoreactivity in the cytoplasm.

Fig5, Fig6, Fig7: Immunohistochemistry localization of DA D2 receptor in human placenta. Both the syncytiotrophoblast and trophoblast showed DA D2 receptor immunoreactivity.

Fig 8: When the primary antibody was substituted by antibody solution buffer, no positive staining was found in any section

Fig 5

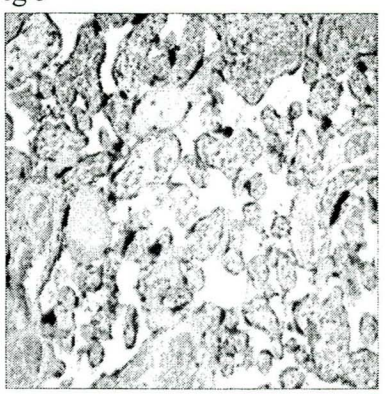


Fig6

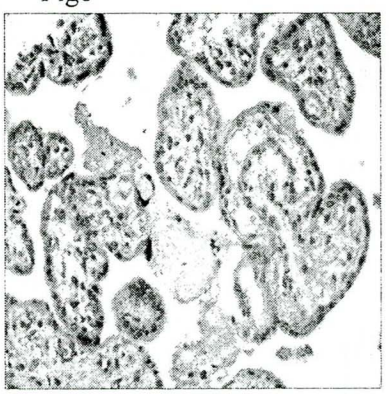


Fig7

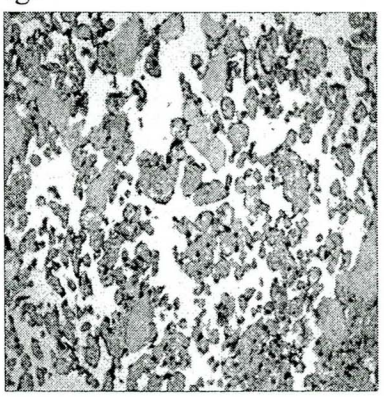
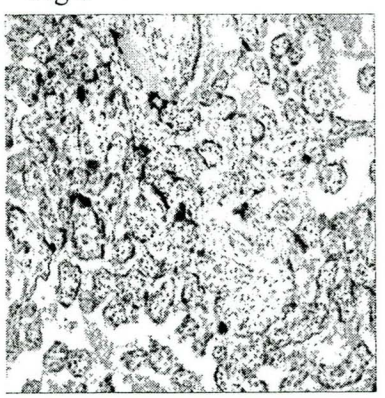


Fig 8



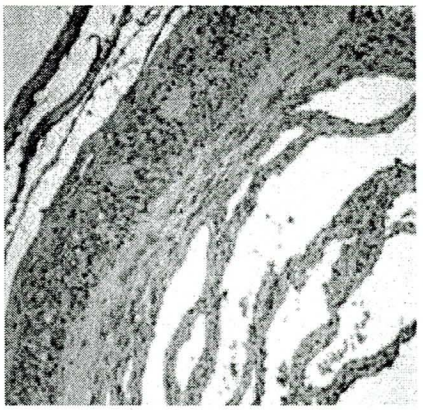
3. Localization of Dopamine in the fetal membranes.

Positive Dopamine staining was found in the amniotic epithelium, reticular layer of chorion, trophoblast layer of the chorion, invasive trophoblast and deciduas cells in fetal membranes.

Strong positive staining was showed in the trophoblast layer of chorion and invasive trophoblast cells in deciduas.

Fig 9: (Magnification, x100) Fig 10: (Magnification, x200)

Localization of Dopamine in fetal membranes, immunoreactivity in the amniotic epithelium, , trophoblast layer of the chorion, invasive trophoblast and deciduas cells.



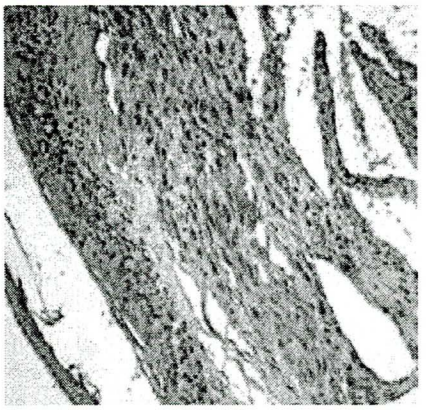
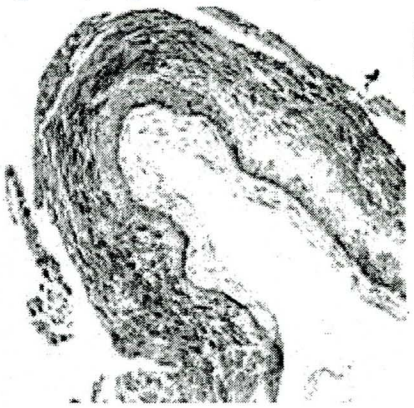
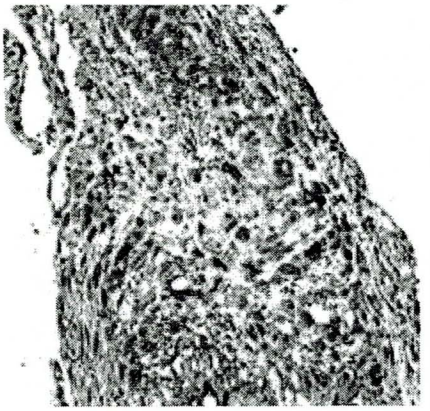


Fig 11: (Magnification, x40) Fig 12: (Magnification, x200)





4. Localization of Dopamine D2 receptor in the fetal membranes

Dopamine D2 receptor immunoreactivity in the amniotic epithelium, trophoblast layer of chorion, invasive trophoblast and deciduas cells in fetal membranes obtained at term.

Strong positive staining was showed in the trophoblast layer of chorion and invasive trophoblast cells in deciduas.

Fig 13, Fig 14, Fig 15, Fig.16: Staining Dopamine D2 receptor was detected in amniotic epithelium, trophoblast layer of chorion, invasive trophoblast and deciduas cells, but immunoreactivity was stronger in trophoblast layer of chorion, invasive trophoblast and deciduas cells.

Fig 13: (Magnification, x100)

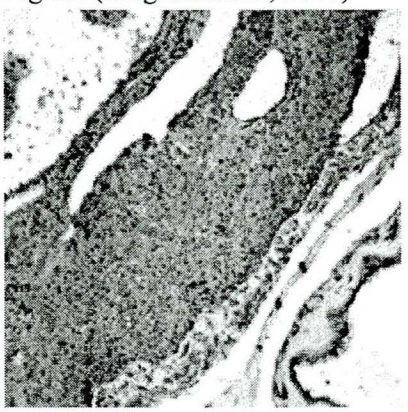


Fig 14: (Magnification, x100)

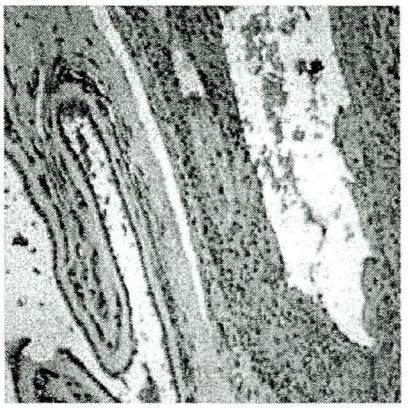


Fig 15: (Magnification, x40)

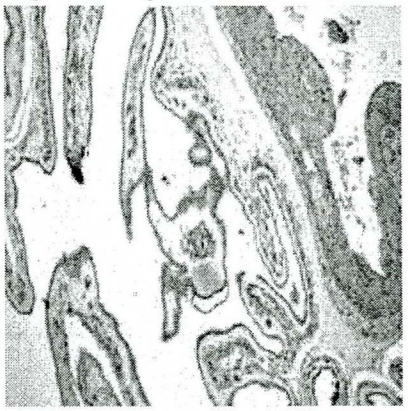
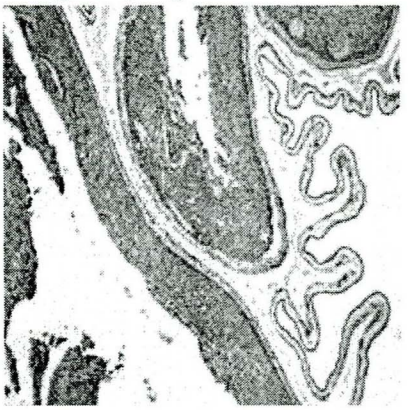


Fig 16: (Magnification, x40)



5. Localization of Dopamine and Dopamine D2 receptor in the Umbilical cords

- Localization of Dopamine was found in the amniotic epithelium and in the Warton's jelly cell,but in the musculature of the umbilical vessel no staining.
- Localization of Dopamine D2 receptor shown in the amniotic epithelium and in the Warton's jelly cell, but in the musculature of the umbilical vessel weakly staining.

Fig 17. Distribution of Dopamine amniotic epithelium, cell in the Wharton's jelly, epithelium of the vessel, but no staining in the vessel musculature.
Fig 18: Dopamine D2 receptor was proved in the Wharton's jelly cell, epithelium of the vessel, and weakly staining in the vessel musculature of the umbilical cords.

Fig 17: Localization of DA in the U. cord
(Magnification, x40)

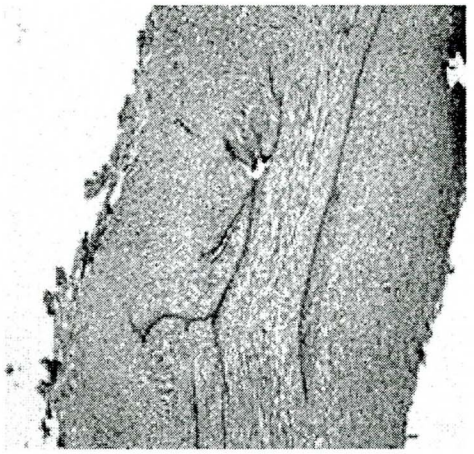


Fig 18: Localization of DA D2 receptor(Magnification, x100)

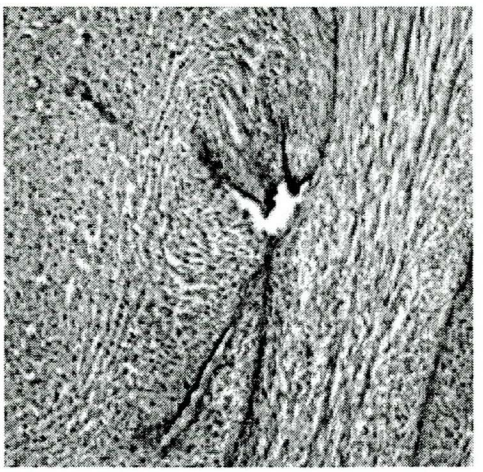


Table 2:

Results of Dopamine immunoreactivity in human placenta, fetal membrane and umbilical cords.

	DA	Results	
		Positive	Negative
Placentae	42	14 (33.3%)	28
Fetal membranes	20	5 (25.0%)	15
Umbilical cords	8	3 (38.5%)	5
Total	70	22	48

Table 3:

Results of Dopamine D2 receptor immunoreactivity in human placenta, fetal membrane and umbilical cords.

	D2 R	Results	
		Positive	Negative
Placentae	50	24 (48.0%)	26
Fetal membranes	25	14 (48.0%)	11
Umbilical cords	12	6 (50.0%)	6
Total	87	44	43

IV. DISCUSSION

The hormones secreted by the villous syncytiotrophoblast are critical for maintaining pregnancy. Early in gestation, human chorionic gonadotrophin (hCG) is essential tomaintain corpus luteum progesterone production. Near the end of the first trimester, the mass of villous syncytiotrophoblast is large enough to make sufficient progesterone and estrogen to maintain the pregnancy. During the third trimester, large quantities of placenta lactogen are produced, hormone purported to have a role as regulator of lipid ancarbohydrate metabolism in the mother. Other syncytiotrophoblast product includespregnancy specific B1- glucoprotein, plasminogen activator inhibition type 2, Growth hormone, collagenases, thrombomodulin, and growth factor receptor…

In vitro experiments have identified several compound which are capable of differentiating cultured cytotrophoblast towards an endocrine phenotype. These include cAMP EGF and hCG itself. Cyclic AMP has been show to up regulate hCG and progesterone secretion. The effect of cAMP in endocrine of human placenta functions has conflicting results. D_2 Dopamine agonist inhibitshuman placenta cAMP production. Moreover dopamine is an endocrine analogy between the human placenta and the hypothalamus-pituitary axis that was proposed some years ago (2)

In this study, we have used immunohistochemistry to identify the Dopamine and Dopamine D_2 receptor in human placenta, fetal membrane, umbilical cords during gestation periods. For the dopamine, the presence of dopamine in the human placenta has already been in between placenta and dopamine, but all most the public articles found the function physiological of dopamine in placenta are dopamine receptors D1, D2 ..etc, not see the report of dopamine localized in placenta.. This is the first time we recognized immunohistochemistry the dopamine in the human placenta..

In the present study, dopamine and dopamine D_2receptor was found in syncytiotrophoblast, stromal cells, capillary endothelium of placental villi, suggesting that human placental villi may produce dopamine and dopamine D_2 receptor

Thereby, the existence of dopamine was confirmed in the placenta and to our knowledge, in the placenta there are some reports that showed the presence of dopamine receptors but it seems few publication to investigate the dopamine in the placenta. It has been reported that the human placenta could produce many neuropeptides (petraglia 1989), growth factors (Holmgren 1992), and cytokines (Berkowitz 1990). These bioactive material maternal might play important roles in placental and fetal development through autocrine, paracrine and endocrine actions.

Monoamine, a bioactive amine, has a wide rang of biological effects on organisms. It was well known that catecholamine concentrations in amniotic fluid increases with the progression of gestation and dopamine concentration increases markedly at the end of gestation compared with nor-epinephrine and epinephrine. Although dopamine is a small and relative simple molecule, it fulfills many diverse functions. The presence of dopamine was not only in the amniotic fluid (2, 13) but also in the human deciduals (7, 9, 19), in the vascular endothelial cells (8), in the placenta membrane vesicles (18, 14), and in the placenta (6, 12, 15). The functional significance of placental dopamine uptakes is currently a matter of conjecture.

In the 1990s, C. Vaillancourt, A. Petit et al reported the Dopamine D_2 receptor was localized in trophoblast of chorion villi using a ligand binding assay and they regulated the release of human placenta lactogen (hCG) via inhibition of adenosine 3',5' cyclic monophosphate camp production, they confirmed the functional correlation existing between the cytotrophoblast syncytiotrophoblast and the hypothalamo-pituitary axis, since in these two systems, dopamine agonist inhibited cAMP production via the D2 receptor subtype (2).

The same authors, in other articles they demonstrated the association of human placenta D_2 receptor with G protein using pertussis toxin sensitive during pregnancies. This reinforces the functional correlation between human placenta and the pituitary (4). And also they have the research " _expression of human placenta D_2 dopamine receptor during

normal and abnormal pregnancies", it was demonstrated the existence of mRNA which encodes a functional D_2receptor and it corresponding membrane protein in human placenta (5), and one other report showed that the dopamine mediated inhibition of all-stimulated ins P in human placenta is not primary event triggered by D_2 receptor stimulated ins P in human placenta is not primary event triggered by D_2receptor Other previous reports, different functions of dopamine and this receptors might be proposed, in a normal pregnancy, the synthesis of hPL increases until the 34 weeks of pregnancy, and the rate of synthesis decreases until parturition, and one of the function of dopamine and this receptor in the placenta is an inhibition on the hPL production of trophoblastic cells.

The dopamine receptor-expressing zone varies according to placental development. Dopamine receptor is mainly expressed in the junction- zone, which is a major area of placental lactogen secretion. Dopamine was also identified in the labyrinth zone of fully development placenta. (15)
On the other hand, Lauren P. Shearman and Jerrold S. Meyer, in the Norepinephrin transporters in the rat placenta with [3H] nisoxetine, thought also may be a few of dopamine transporters in the rat placenta, but they could not rule out the possibility of a very low level of dopamine transporter _expression (11). To evaluate the physiological role of dopamine Fugimi Arai, YasuoKishimoto et al proved the presence of D_2 receptor in human placenta, dopamine might stimulate the production of prostaglandin in human decidual via a mechanism that is mediated by dopamine D_2 receptor.(7)

Hyun Jon Kim, Phil Ok et al, in the Localization of dopamine D_2receptor mRNA in the human placenta and the anti-angiogenic effect of Apomorphine in the chorioallantoic membrane, confirmed by in situ hybridization,the presence of dopamine D2 receptor in trophoblast cell of chorionic villi, and in vascular endothelial cell of small and large stem villi. Frome these result, they suggested that dopamine might influence the neo-vascularization of placenta tissue though dopamine D2 receptor during development of pregnancy (8).

M.A.Elwan et al demonstrated that human placenta amniotic Epithelial cell express Dopamine D2 Receptor mRNA. Sequencing of the amplified PCR fragment showed 100 per cent homology with the Dopamine D_2 Receptor of the human brain used as positive control. The physiologic significance of the presence of D_2 receptors in human placenta amniotic epithelial cell is currently unclear. However, Dopamine receptor has been reported to be present in human placenta where they play an important role in controlling the fetal milieu and the process of parturition (14).

Functionally, stimulation of traditional D_2receptors is generally thought to inhibit adenylate cyclase activity via interactions with G proteins. Indeed, a variety of studies have demonstrated that transfect recombinant D_2 receptors can inhibit adenylase cyclase in many cell types. The effects of dopamine receptor activation in signal transduction mechanism differ between the neuronal and fibroblast cell lines, and D_2 receptors are inhibitory in both cell types (22), in human placenta, the presence of ß-adrenergic receptors in basal membrane close to the fetal circulation, The relatively high doses of dopamine necessary to stimulate adenylate cyclase could be the consequence of the high proteolytic activites of the human term placenta, especially towards catecholamines. The inverse relationship reported between dopamine and prolactin in human amniotic fluid at the end of pregnancy suggested an inhibitory effect of dopamine on decidual prolactin production, similar to one observed in pituitary gland. (6) In our study, we used only the normal placentae at term, so we can not observe the difference between dopamine and dopamine D_2 receptor in normal pregnancy and pathologic pregnancy, also the change of intensity reaction in pregnancy progress. But according to C. vaillancourt (5), there are the variation of abnormal pregnancy, the relative placenta level of D_2 receptor were decreased in human pre-eclampsia pregnancy, compare with those in placentae from normal pregnancies, also they remarked the relative level of D_2 receptor protein in human placentae from hydatiform moles was lower than what observed in normal placenta at the same period of pregnancy. We was also remarkable, in our experiment, between

dopamine and dopamine receptor D_2 immunoreactivity of dopamine receptor D_2 was more sensible than dopamine (table 2, 3) .

For instance, Dopamine stimulates human placenta progresterone production (Battisa 1990), inhibits the releases of placenta prolactin and growth hormone (2), (16), (Petit 1990) and increases placental synthesis and releases of prostaglandin (9), (19). Also, Dopamine receptors may be involved in the regulation of placental angiogenesis and vascularization (8), moreover, the level of _expression of D_2 receptor in human placenta varies during different stages of gestation and also differs between normal and abnormal pregnancies indicating an important role of Dopamine in placental function (5). Finally, another possible function of the placenta-Nor-epinephrine may be to regulatethe availability of catecholamine to stimulate placenta ß-adrenergic receptor. Various studies have demonstrated the present of ß-receptor as well as ß-receptor-sensitive adenylyl cyclase in the human placenta.

In this study, we observed that syncytiotrophoblast cells not only produced Dopamine but also contained the Dopamine D_2 receptor, suggesting that trophoblast cells producing dopamine and dopamine D_2 receptor may take part the role in human placentae through paracrine and autocrine interactions.

In our report, we proved the immuno- positive of dopamine and dopamine D_2 receptor in fetal membranes, this result coincided with previous articles indicated that dopamine in human amniotic fluid is biological active as judge by as ability to inhibit rat pituitary prolactin secretion, because amniotic fluid is in direct contact fetal membranes, so may be the function of fetal membranes like functions of amniotic fluid (katsuhito kada 1991) (9).

Fetal membranes consist of amnion, chorion and decidual, prostaglandin produced in decidua which is in direct contact with uterine muscle tissue, are considered to cause uterine muscle contraction.

The presence of dopamine in fetal membranes was stimulated the synthesis of prostaglandin by human decidua , and DA increased amount in amniotic

fluid during late gestation and high concentration markedly at the end of pregnancy relate to human parturition. Prostaglandin PG-E_2, PGF_2 mainly produced by fetal membranes. PG-E_2, PGF_2have actively involved in the initiation and / or maintenance of human parturition.

The role for fetal dopamine in the onset of parturition is presently considered since dopamine stimulates human uterine activity at term pregnancy, very intriguing is the role of fetal dopamine in the prolactin synthesis by the deciduas.

V. CONCLUSION

In summary, this report demonstrated the localization of dopamine immunoreactive in the human placenta , fetal membranes, and umbilical cords at full term, including the syncytiotrophoblast, trophoblast cells, and decidua vera. We are the first person who showed the localization of Dopamine in the placenta.

The placenta not only produced Dopamine but also contained the Dopamine D2 receptor, suggesting that syncytiotrophoblast, trophoblast cells, decidua producing dopamine and dopamine D2 receptor and may be they take part the role in human placenta through paracrine and autocrine interactions.

We propose the hypothesis that placenta secreted dopamine and also dopamine D_2 receptor, that have closely the relationship with placenta. So placenta may be the source of both Dopamine and Dopamine D2 receptor.

Up to now, although the physiologic significance of Dopamine in the placenta is still unclear, but many functions of DA were defined as stimulated human placenta progesteron production (Battista 1990), stimulated the production of prostaglandin in human decidua in fetal membranes at term (7, 9, 19), synthesis regulated placental lactogen. It is speculated that DA of maternal could influence growth of placenta and fetus via the long form of Dopamine receptor systems in syncytiotrophoblast, trophoblast cells, decidua.

According to our results and previous studies, Dopamine and Dopamine receptor have many functions useful to the organs in the body and can be secreted out of the brain, placenta was one of the origin where can secrete Dopamine and Dopamine receptor, so we suggest that may be, in the future, we can apply the placenta to produce and synthesize the dopamine and dopamine receptor to treat the diseases especially in the field of Obstetrics and Gynecology.

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Pictures legend

Localization of Dopamine in placenta

Fig 1

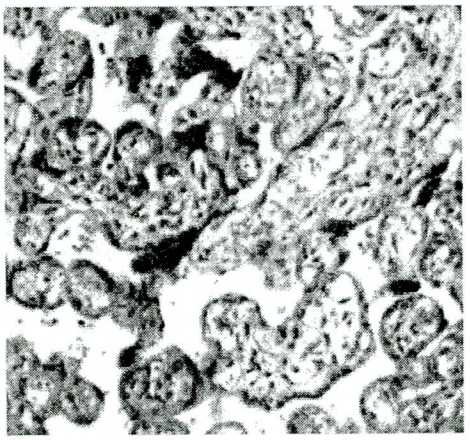


Fig 2

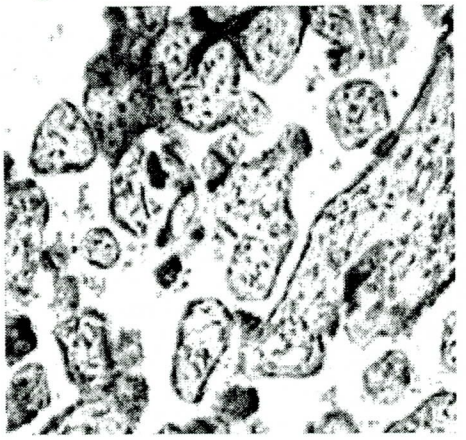


Fig 3

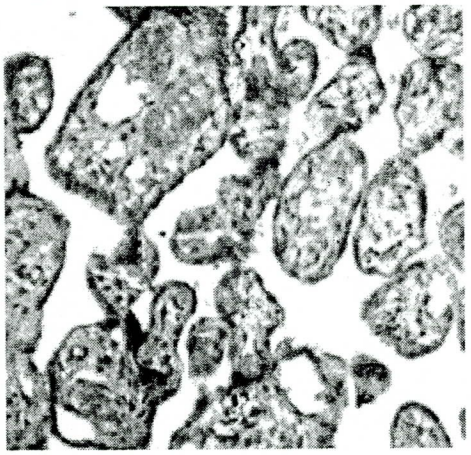
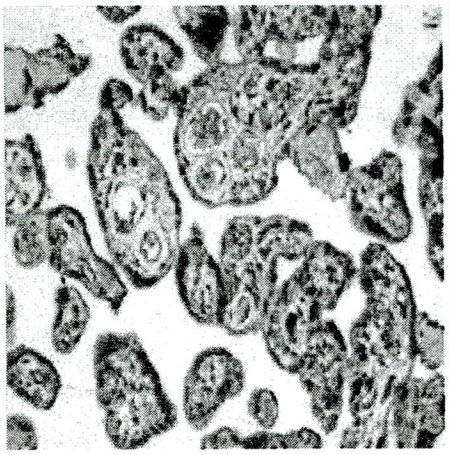


Fig 4

Pictures legend of Dopamine D2 receptor in the placenta

Fig 5



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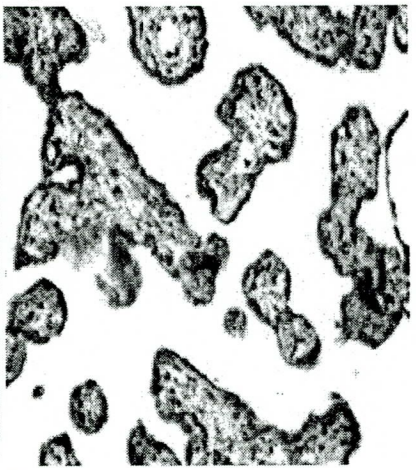
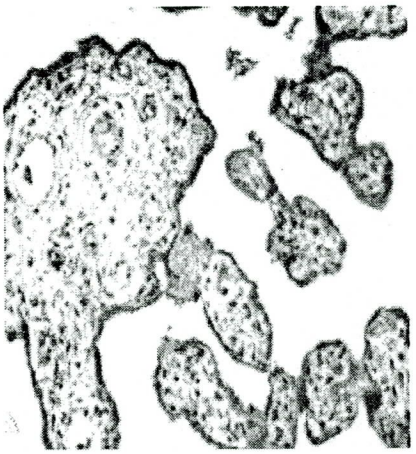


Fig 7

Fig 8



Pictures legend

Localization of DA D2 receptor in fetal membranes

Fig 9

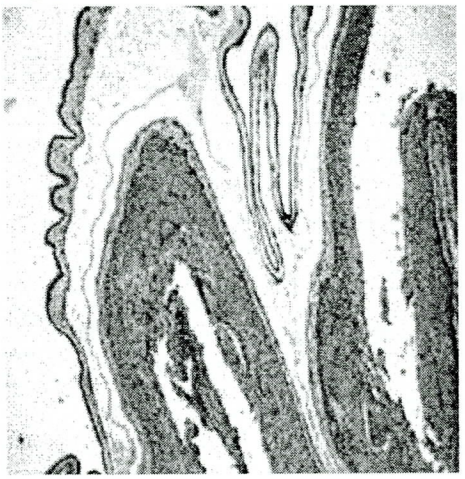


Fig 10

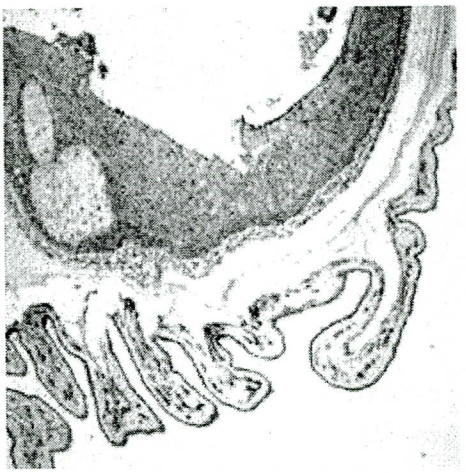


Fig 11

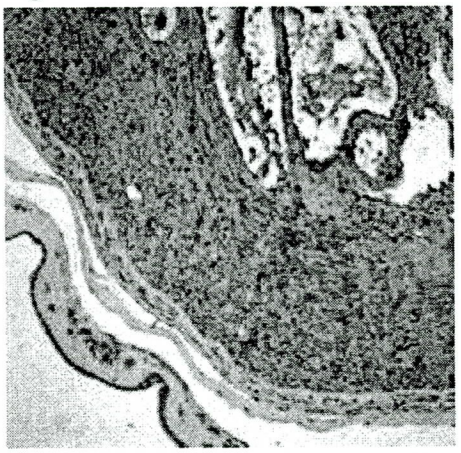


Fig 12.