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Effect of Platelet-Activating Factor on Cyclic Nucleotide Level in Rat Uterine tissue during Preimplantation Period

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흰쥐의 임신초기에 있어서 자궁 조직중 Cyclic Nucleotide의 변화 및 Platelet-Activating Factor의 영향에 관한 연구

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=국문초록=

본 연구의 목적은 임신 초기 자궁 조직중의 cyclic nucleotide의 변화 및 PAF가 이들에 미치는 영향을 관찰함으로써 PAF가 흰쥐의 초기 임신에 어떻게 관련하는지를 조사하기 위함이다.

시험구로써 임신 각 일에 1㎍의 PAF 혹은 이것의 수용체 길항제인 1.25mg의 BN-52021이 근육내 조사되었고 비 임신구 및 대조구에 대하여는 PBS만이 주사되었다. 자궁 조직중의 cAMP 및 cGMP 농도는 분석용 test kit를 사용하여 분석되었다.

비 임신구 경우 자궁 조직중 cAMP 농도는 단백질 mg당 2.91±0.33 pmol로서 임신구 보다도 낮았고 cGMP 농도 또한 0.39±0.20 pmol로서 임신구보다 낮은 경향이었다.

자궁 조직중 cAMP의 최고농도는 임신 3일째(5.92±1.72 pmol/mg protein)였고 cGMP 경우는 임신 4일째(1.03±0.22 pmol/mg protein)이었다.

임신 각일에 PAF는 PAF처리하지 아니한 대조구에 비하여 증가된 cAMP를 보여주었으나(임신 0, 2, 그리고 4일째 경우 $p<0.05$) BN-52021은 감소된 경향을 나타내었다. cGMP에 대하여는 PAF나 BN-52021 공히 일정한 효과적 경향을 보이지 아니하였다. 따라서, 임신은 자궁 조직중 cyclic nucleotide에 영향을 미칠 수 있으며 흰쥐의 착상기동안 PAF는 cGMP에 대하여 보다는 cAMP에 영향을 미침으로써 착상에 관련된 일련의 반응에 영향을 미칠 것으로 사료된다.

Key Words: Platelet-activating factor(PAF), Cyclic nucleotides, Implantation.

INTRODUCTION

Platelet-activating factor (PAF) is known to be phospholipid which is produced by basophils, neutrophils, platelets, macrophages, endothelial cells and isolated tissue preparation, and mediate platelet-related events to include inflammatory and vasoactive responses (Braquet et al., 1987).

Previous studies suggested that platelet-activating factor(PAF) played a major role in rat implantation(Smith and Kelly, 1988; Acker et al., 1989; Park and Kwun, 1991). But the specific mechanism of PAF-related responses in implantation process is not yet cleared.

Steroids and prostaglandins that play a

major role in implantation may have an effect on this process by cyclic nucleotides-mediated mechanism. Estrogens act on the uterine target tissue through the first and second messenger system in which cAMP is the secod messenger(Robison et al., 1968). The administration of estrogens stimulated uterine adenyl cyclase activity(Rosenfeld and O'Malley, 1970) and increases the uterine level of cAMP(Szego and Davis, 1967). There is also a report that estrogen and the counter action of progesterone combine to play a central role in regulating uterine prostaglandins(PGs) as well as regulating cyclic nucleotides(Kuhl et al., 1974). Little is known of the precise mechanism by which PGs have their effects within the endometrium. There are reports that PGE alter the activity of the adenylate cyclase and the resultant change in the intracellular level of cAMP so that PGE have their biological effects(Mc Mahon, 1974;Kuehl et al., 1976;Singhal et al., 1976). With the findings that PGE level is elevated at implantation sites(Kennedy, 1977) and at the same time cAMP concentration is also elevated there(Vilar-Rojas et al., 1982). Kennedy(1983) suggested that effect of PGE_2 on endometrial vascular permeability in the rat uterus be mediated by cAMP. Studies on the local and hormonal mechanisms that trigger endometrial differentiation during the preimplantation period suggest that cyclic nucleotide mey be involved in these mechanismt the intracellular level(Sim, 1974;Rankin et al., 1977).

Cyclic nucleotides are involved in almost all aspect of the reproductive process. The specificity of the requirement for cAMP in implantation-related reaction has been questioned (Leroy et al., 1974;Webb, 1975a, 1975b;Fernandez-Noval and Leroy, 1978). Leroy et al. (1974) insisted that the intrauterine administration of cAMP itself could not induce decidual reaction. But Webb(1975a) suggested that dcAMP induced implantation of diapausing mouse blastocysts in the abscence of estrogen, and a single intraluminal injection of dcAMP promoted cell division in the uterine stroma and sensitized the endometrium(Webb, 1977). Fernandez-Noval and Leroy(1978) reported that a normal decidual reaction, indicating uterine sensitization, was observed when embryo in the uteri of ovariectomized mice treated with progesterone was implanted after intraluminal injection of cAMP. They reported additionally that the intraluminal administration of cAMP in mice during delayed implantation induced the endometrium/blastocyst interaction.

Dey et al., (1978) reported that the level of cAMP and cGMP changed shortly before implantation in the rabbit. Kennedy(1983) reported that agents such as cholera toxin which was known to increase cAMP levels were able to induce the decidual reaction. According to the observation by Fortier et al. (1989) in the rabbit the overall adenylate cyclase activity is increased significantly on day 1 of pseudopregnancy compared to that on day 0(oestrus), however the activity is decreased progressively on day 6.5 and 9 of pregnancy and exhibited a recovery on day 15 of pregnancy. Such a dramatic alteration of adenylate cyclase activity in rabbit endometrium during pseudopregnancy of adenylate cyclase activity in rabbit endometrium during pseudopregnancy or pregnancy suggests a possible involve-ment of cAMP in the regulation of endometri-al changes in early pregnancy process.

As shown in the above-mentioned reports there are possibilities that cyclic nucleotides, particularly cAMP, are involved in the process associated with endometrial changes during the period of pregnancy, and especially in the other events including implantation in the rat. However most of the works insist that cyclic nucleotides only mediate the action of substances essential to the process of implan-

tation rather than the administration of cyclic nucleotides itself can induce such reaction (Leroy et al., 1974;Webb, 1975a, 1975b;Hoffman et al., 1977;Rankin et al., 1977;Kennedy, 1983.)

It is therefore possible that PAF which is participated in the production(Braquet et al., 1987;Smith and Kelly, 1988;Rabinovici and Angle, 1991) and response(Alecozay et al., 1991) of these substances and play a same action as such substances in implantation response may also act through a cyclic nucle otides-mediated mechanism directly or indirectly.

The purpose of this investigation is to observe the change in the level of cyclic nucleotide in uterine tissues and the effect of the PAF on cyclic nucleotides in the early pregnant rat, in order to better understand the possible reciprocal relat ionship betweeu cyclic nucleotides and PAF in early pregnancy.

MATERIALS AND METHODS

1. Animals:

Sprague-Dawley female rats weighing 180 -200g (YuHAN Co, Ltd.) were used throughout the experiments. Each group contains seven to thirteen of rats

Day 0 of gestation was defined by present of copulatory plugs and spermarozoa in the vaginal tract.

2. Reagents:

PAF(L-α-Phosphatidylcholine, β-Acetyl-r-0-Alkyl) was purchased from. Sigma Chemical(St. Luis, MO, USA) and BN-52021, a specific PAF antagonist(Braquet et al., 1985; 1987) was kindly gifted from I.H.B. Res. Laboratories(Le Plessis-Robinson, France). Both were stored at -70℃. For use in injection experiments, dilutions of PAF and BN-52021 were made in phophate-buffered saline(PBS; 0.02 M-NaPi;0.09% W/V NaCl, pH 7.4) containing 0.25% bovine serum albumin(BSA fraction V powder:Sigma Chemical). Cylclic nucleotides assay kits were purchased from Amersham International.(England) and were used for the measurement of cyclic nucleotides.

In this assay deionized distilled water(Ca^{2+} & Mg^{2+}-free) and 0.05M Tris-EDTA buffer (pH 7.4) containing 4mM EDTA were used.

3. Experimental design

Rats were devided into test and control group. On day 0, 1, 2, 3, 4 or 5 of pregnancy, the test groups were intramuscularly injected with 1μg of PAF per 200g body weight, or 1. 25mg of BN-52021 per 200g body weight while the control groups were injected with PBS in a total volume of 100μl of the solution containing these substances, or solvent alone as control. Fifteen minutes after treatment, the uteri were removed and weighed as wet weight. The samples were homogenized(20% W/V) by polytron homogenizer (PT 10ST, a pulse frequency of 7300 Hz, 30-60 sec) in ice -cold 0.05M Tris-EDTA buffer(pH 7.4), containing 4mM EDTA, and were centrifuged at 800g for 10min at 0℃. The residuals were rehomogenized and were recentrifuged under same conditions. The supernatant solutions were removed and were assayed for protein measurement by the method of Lowry et al. (1951), followed by heating for 3 min in boiling water bath to coagulate the protein. After centrifuation at 3,000g for 15min at 0℃, the supernatant solutions were removed and were frozen in a solid CO_2-acetone mixture, and were then stored at -70℃ until the assay was performed.

The collected supernatant solutions were ex-tracted three times with 5ml of ether saturated with H_2O. The aquous phases were dried and were reconstituted with 1ml of Tris-EDTA buffer.

The concentrations of cyclic cAMP and

cGMP in uterine tissue were assayed in duplicate with cyclic nucleotide test kits(Amersham, England).

4. Assay validation

Standard curves showed that the methods were accurate at 1.0-16.0 pmols per tube for cAMP assay and 0.5-8.0 pmols per tube for cGMP. The samples were diluted so that only the linear portion of the curve was used. The sensitivities of the assay of cAMP and cGMP were 0.05pmol and 0.04 pmol respectively.

In order to estimate the recovery and accuracy of assay sample was spiked several concentrations of cAMP and cGMP and 5 aliquots of each concentration(1ml per aliquot) were purified as described above. The dried extracts were re-constituted in 1ml of as-say buffer. The results obtained from assays showed the recoveries of 87.1-101.3 percent for cAMP and 92.7-106.5 percent for cGMP as shown in Table 1, 2.

Table 1. Recovery of cAMP assay in uterine tissue

cAMP added (pmol/ml)	Average cAMP measured (pmol/ml)	Recovered (pmol/ml)	Mean recovery (%)
0	43.7	—	—
34.0	78.1	34.4	101.3
64.0	105.5	61.8	96.5
256.0	266.7	223.0	87.1

The assayed result show the sensitivity of 1.0 pmol and is validated in the range 20-320 pmol/ml of extract.

Table 2. Recovery for c-AMP assay in uterine tissue

cAMP added (pmol/ml)	Average cAMP measured (pmol/ml)	Recovered (pmol/ml)	Mean recovery (%)
0	5.9	—	—
4.4	10.4	4.5	103.1
8.8	15.3	9.4	106.5
35.2	38.5	32.6	92.7

The assayed result show the sensitivity of 0.4 pmol and is validated in the range 5-80 pmol/ml of extract.

RESULTS

1. The level in cyclic nucleotide in uterine tissue during peri-implantation period

1) The level of cAMP

Figure 1 shows the profile of cyclic mecleotides during early pregnancy.

The level of cAMP in uterine tissue of non-pregnant rat at pro-oestrus was 2.91 ± 0.33 pmol/mg protein, while the level of cAMP in uterine tissue of pregnant rats were 4.00 ± 1.57, 2.74 ± 0.31, 4.11 ± 1.27, 5.92 ± 1.72, 4.78 ± 0.36 and 3.60 ± 0.32 pmol/mg protein on day 0, 1, 2, 3, 4 and 5 of pregnancy, respectively, being significanthy higher($p<0.05$) than that of non-pregnant rat except on day 1 of pregnancy. This result indicated that pregnancy affected cAMP level in uterine tissue, reaching maximum level of 5.92 ± 1.72 and 4.78 ± 0.36 pmol/mg protein on day 3 and day 4, respectively(Table 3, Fig. 1).

2) The level of in cGMP

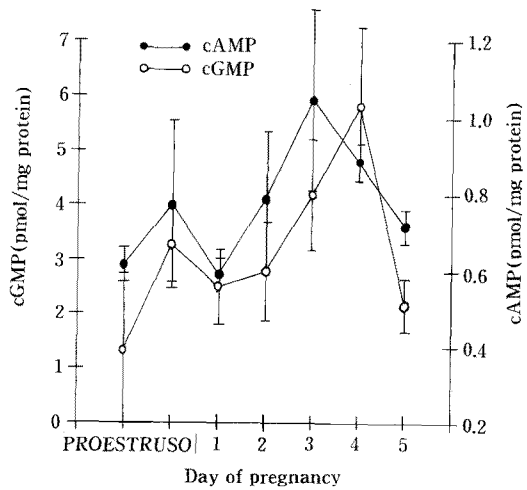

Fig. 1. Changes in uterine cyclic nucleotide levels during early pregnancy. The c AMP and c GMP contents in uterus of non-pregnant rat(pro-oestrus) were 2.91 ± 0.33 and 0.39 ± 0.20 pmol/mg protein, respectively.

Table 3. The level of cAMP on each day of pregnancy in rat uterine tissue

(pmol/mg protein)

Day / Treatment	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
BN-52021	2.73±0.85	1.69±0.91[c]	3.95±0.88	2.32±0.71[c]	3.45±1.00	2.45±0.98[c]
Intact	4.00±1.57[a]	2.74±0.31[b]	4.11±1.27[a]	5.92±1.72	4.78±0.36[a]	3.60±0.32
PAF	8.21±3.19[b]	3.86±1.12	7.30±2.63[b]	6.38±1.41[b]	6.68±2.14[b]	5.65±3.40[b]

Day 0 is defined as day of the presence of spermatozoa and values are mean±SD of 7-13 observations.
Values of $p<0.05$ were taken as significant.
Values with the different superscript are signicantly differnt from each other.

Table 4. The level of cGMP on various days of pregnancy in rat uterine tissue

(pmol/mg protein)

Day / Treatment	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
BN-52021	0.80±0.23	0.51±0.09	0.70±0.29	0.76±0.19	1.19±0.25[c]	0.51±0.04
Intact	0.67±0.11[a]	0.56±0.09	0.60±0.13[a]	0.80±0.17	1.03±0.22	0.51±0.07[a]
PAF	0.90±0.22[b]	0.53±0.21	0.41±0.10[b]	0.64±0.19	0.85±0.32[b]	0.33±0.11[b]

Day 0 is defined as day of the presence of spermatozoa and values are mean±SD of 7-13 observations.
Values of $p<0.05$ were taken as significant.
Values with the different superscript were signicantly differnt from each other.

The cGMP content in uterine tissue of non-pregnant rat at pro-estrus was 0.39±0.20 pmol/mg protein. The result of time course of cGMP level during first 6 days after pregnancy recorded 0.67±0.11, 0.56±0.09, 0.60±0.13, 0.80±0.17, 1.03±0.22 and 0.51±0.07 pmol/mg protein on each day of pregnancy and the level as slightly higher than that of non-pregnant rat. The highest cGMP level of 0.80±0.17 on day 3 and 1.03±0.22pmol/mg protein on day 4 were observed in uterine tissues as shown in Table 4 and Fig 1 which are similar tendency to change in cAMP levels.

2. Effect of PAF on cyclic nucleotide level in utericne tissue

1) Effect of PAF on cAMP level

Table 3 illustrate the effects of PAF(1μg) on uterine cAMP level in the rat.

Administration of PAF induced the increased level of cAMP compared with those of intact pregnant rats(8.21±3.19 VS 4.00±1.57on day 0;3.86±1.12 VS 2.74±0.31on day 1;7.30±2.63 VS 4.11±1.27on day 2;6.38±1.41 VS 5.92±1.72 on day 3;6.68±2.14 VS 4.78±0.36 on day 4;5.65±3.46 VS 3.60±0.32 on day 5). Rats treated on day 0, 2, and 5 of pregnancy showed the significantly increased concentrations of cAMP($p<0.05$).

2) Effect of PAF on cyclic GMP level

When PAF was administered to pregnant rats as shown in table 4, the result indicated that the tendency of changes in cGMP level was not consistent, compared with those of in-tact pregant rats.

3. Effect of BN-52021 on cyclic nucleotide level in uterine tissue

1) Effect of BN-52021 on cyclic AMP level

As shown in Table 3, BN-52021 treatment elicited the decreased cAMP level although it was not significant except on day 1 of pregnancy 1, compared with those of intact pregnant rats.

2) Effect of BN-52021 on cGMP level

The result obtained from the administration of BN-52021 did not show any, consistent

tendency of change in cGMP level compared with those of intact pregnant rats similaly to that by PAF treatment(Table 4).

DISCUSSION

The levels of cAMP and cGMP in uterine tissues were higher in pregnant rats than in non-pregnant rats(pro-oestrus), implying that cyclic nucleotide levels in uterine tissue can be influenced by pregnancy.

Cyclic cAMP showed peak level on day 3(5.92±1.72 pmole/mg protein) and cGMP on day 4(1.03±0.22 pmole/mg, protein). These data suggest that cyclic nucleotides are related to implantation in the rat. On each day of early pregnancy PAF induced increased cAMP level in uterine tissue compared to those of intact rats. However the result did not show the consistent tendency in the influence on the level of cGMP. PAF antagonist, BN-52021 induceddecreased cAMP level, and showed the similar pattern to the influence of PAF on cGMP level. This result indicate that PAF influence cAMP level in uterine tissue rather than cGMP level during peri-implantation period. As already described in other report(Parkond Kwun 1991), PAF might play a major role in implantation. These results conjugated with this report may be demonstrate a possible involvement of PAF in the regulation of peri-implantation through a cAMP-mediated process.

There are many reports which decribed the effects of cyclic nucleotides on the decidual reaction or blastocyst implantation(Leroy et al., 1974;Webb, 1975, 1977;Fernandez-Noval and Leroy, 1978).

As well known, estrogen, progesterone, prostaglandins(PGs), PGE_2 in particular, and leukotriens play a important role in a series of events related to implantation. as PAF does. For example, these substances are related to trigger the responses prior to implantation, such as increased vascular permeability, increased sensitivity, and decidual reaction in the uterine endometrium. In general, these substances increase cAMP in such a specific situation or act through a cAMP-mediated process(Robinson et al., 1968). In this regard Rosenfeld and O'Malley(1970) reported that estrogen actually activated adenylate cyclase in the castrate rat uterus and progesterone produced a delayed stimulation of oviduct adenyl cyclase. In the other hand Wolfe and Shulman(1969), Remold-O'Donnell(1974), and Gemsa et al.(1975) suggested that prostaglandins activate adenylate cyclase in a number of cell systems. Also Rankin et al.(1977) insisted that PGE_2 induced the decidual reaction through a mechanism mediated by cAMP. Fortier et al.(1987) have found that adenylate cyclase activity was stimulated by PGE_2 to a greater extent in cultured stromal cells than in epithelial cells in rabbit endometrium. Resently Fortier et al.(1989) insisted a possible involvement of PGE_2 in the regulation of endometrial receptivity through a cAMP-mediated process, suggesting that the stroma appears to be the target for PGE_2 action. Furthermore the PGE_2 response in cultured cells is increased after progesterone treatment in the rabbit.

There are more reports that the role of prostaglandins are mediated by cAMP(Tepperman and Soper, 1981;Kennedy, 1983;Peterson et al., 1988). Leukotriens have vasomotor properties(Drazen et al., 1980) and exhibit a marked increase on day 3 of pregnancy in the rat.(Malathy et al., 1986). Cyclic cAMP-dependent phosphodiesterase controls cytoplasmic level of cAMP. Chasin and Scott(1978) reported that a selective lipoxygenase inhibitor, FPL-55712 inhibited both cAMP and cGMP phosphodiesterase, PAF also exhibit vasomotor properties(Drazen et al., 1980: Pirotzky et al., 1984) and in the adult rabbit increase vascular permeability(Humphrey et

al., 1982;1984;Angle et al., 1986), influencing decidual reaction(Acker et al., 1989).

Previously Ham et al.(1975) and Neal et al. (1976), and Peleg(1983) have reported the relationship between estrogen and PGs, cyclic nucleotides and between PGs and progesterone, respectively. Further PAF caused a dose-dependent increase in the synthesis of PGE_2 by an enriched glandular epithelial cells of mid-secretory endometrium(Shaw et al., 1981;Schlondorff et al., 1984;Billah et al., 1985;Smith and Kelly, 1988). Recently Rabinovici and Angel(1991) reported that PAF induced progesterone secretion in leuteinizing granulosa cells *in vitro*, and Alecozay et al (1991) suggested that in respect to interaction between PAF and prostaglandins, PAF in stromal cells was increased by PGE_2 in the presence of progesterone while PGE from gland cells was increased and PGF decreased by PAF in the presence of oestradiol-17β.

Attentions are attracted by report that it is possible to inhibit the effects of PAF by modulating the level of cyclic nucleotides(Baranes et al., 1986 for cAMP, Chignard et al., 1985 for cGMP). Thus it is directly or indirectly possible that PAF, by the activation of adenylate cyclase, subsequenthy increases intracellular cAMP concentrations, or alternatively the release of any substances, such as steroids, arachidonic acid metabolites thereby modify endometrial function to initiate an increase in endometrial vascular permeability, subsequent decidualization which induce ther implantation of embryo. The hypothetical(Dey and Johnson, 1980) relationship of histamine in implantation may be applicable to the case in this question. In conclusion the action mechanism of PAF through cAMP-mediated process for a regulating role of implantation seems likely to be real whether it is direct or indirect.

The challenge for the immediate future is to identify and to testify this process or mechanism that the action of PAF is mediated by cAMP for a regulating role of implantation.

ABSTRACT

This study was carried out to observe the change in uterine cyclic nucleotide level and the effect of PAF on cyclic nucleotides in uterine tissue in early pregnany in order to understand reciprocal relation ship between PAF and cyclic nucleotides in pregnancy in the rat.

The test groups were injected intramuscularly with 1μg of PAF or 1.25mg of BN-52021 on day 0, 1, 2, 3, 4 and 5 of pregnancy. The level of cyclic nucleotide in removed uterine tissue was assayed by using cyclic nucleotides test kits.

The results showed that the cyclic AMP content in uterine tissue of non-pregnant at pro-oestrus rat was 2.91 ± 0.33 pmol/mg protein which was lower than those of pregnant rat. The cyclic GMP content in uterine tissue of non-pregnant rat was 0.39 ± 0.20 pmol/mg pro-tein which was also lower than those of pregnant rats. The maximum level in cAMP was 5.92 ± 1.72 pmol/mg protein on day 3 and cGMP, 1.03 ± 0.22 pmol/mg protein on day 4.

On each day of pregnancy, PAF induced the increased cAMP level compared with that of intact rat. That was significant on day 0, 2 and 4 of pregnancy, $p<0.05$, on the other hand PAF receptor antagonist, BN-52021 decreased cAMP level in uterine tisssue.

PAF as well as BN-52021 had not an consistent effect on changes in cGMP level.

These results suggest that cyclic nucleotide levels in uterine tissue ware increased during early pregnancy and PAF influences cAMP level in uterine rather than cGMP level during peri-implantation period, accordingly demonstrating a possible involvement of PAF in the regulation of implantation-related events through cAMP-mediated process.

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