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EFFECT OF DAY OF PREGNANCY AND PREGNANCY TERMINATING AGENTS ON PROSTAGLANDIN SYNTHESIS AND METABOLISM AND HISTAMINE METABOLISM IN THE RAT UTERO-PLACENTAL FETAL COMPLEX AND LUNG

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SUMMARY

Synthesis and metabolism of prostaglandins (PG) in the rat placenta during the first two-thirds of pregnancy is low with the exception of a large peak of activity on day 11. During the last third of pregnancy placenta PG-synthesis is low but PG-metabolism increases sharply. In contrast, uterine PG synthesis is low during the first two-thirds of gestation but increases during the remainder of pregnancy, peaking at parturition. Uterine PG-metabolism is low throughout pregnancy. Fluctuation in sex steroid levels during pregnancy may be related to changes in PG-synthesis and metabolism. Maintenance of vital functions in pregnancy may require timed changes in prostaglandin as well as hormonal balance. The non-hormonal non-prostaglandin compounds L-10503 (2-(3-methoxyphenyl)-5,6-dihydro-s-triazole [5,1-a]-isoquinoline) and L-11204 (2-(3-ethoxyphenyl)-5,6-dihydro-s-triazole [5,1-a]-isoquinoline) which terminate pregnancy after blastocyst implantation in the uterus, inhibits PG-metabolism. Administration of these compounds to rats on day 9 of gestation reduces PG-metabolism in the placenta, uterus and lungs whereas treatment on pregnancy days 13 or 15 decreases PG-metabolism only in the maternal and fetal lungs. PG-synthesis is not affected under these conditions. Male and non-pregnant female rats treated with L-10503 and 24 h later given [^{3}H]-PGF$_{2\alpha}$ or [^{3}H]-PGE$_1$, have a reduced ability to metabolize PG as measured in plasma. L-11204 administered on day 9 of pregnancy increased placental, uterine and fetal histamine. Placental histaminase was also increased by this treatment. Alteration of PG and/or histamine metabolism may be involved in the anti-fertility activity of these novel compounds.

INTRODUCTION

The role of prostaglandins in pregnancy has still to be defined. It has been shown, however, that plasma prostaglandins increase at the time of partuition [1, 2]. Moreover alteration in prostaglandin levels may be important in the physiology of pregnancy since they may be involved in ovulation [3, 4], ova transport [5], oviductal motility [6], fertilization [1, 3], uterine vascularization and implantation [7] and fetal support [8] as well as in parturition. Relatively little is known about the levels or balance of prostaglandins at different stages of pregnancy or the enzymatic activity required for the synthesis or metabolism of prostaglandins in the various reproductive tissues. In order to better define the pattern of prostaglandin synthesis and metabolism during pregnancy we analyzed various reproductive tissues in the rat for these end points [9]. The importance of these parameters to the pregnant state was investigated through the use of the nonhormonal compounds L-10503 (2-(3-methoxyphenyl)-5,6-dihydro-s-triazole [5,1-a]-isoquinoline) and L-11204 (2-(3-ethoxyphenyl)-5,6-dihydro-s-triazole [5,1-a]-isoquinoline) (Fig. 1). L-10503 has been shown to terminate pregnancy in various animal species when administered post-coitally [10, 11]. In addition it has been shown that this compound inhibits prostaglandin metabolism in the rat placenta and uterus without altering prostaglandin synthesis [12]. However, prostaglandin metabolism inhibition may not be the only mechanism involved in pregnancy termination by these compounds. In toxemia of pregnancy it has been shown that histamine metabolism is altered [13]. It was therefore of interest to study the effect of drug treatment on histamine and histaminase levels in the placenta and uterus of the rat.

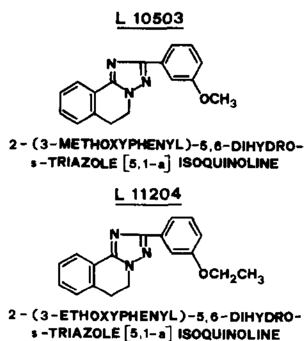

Fig. 1.

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EXPERIMENTAL

Animals. Female rats (Charles River CD-derived from Sprague–Dawley) weighing 200–250 g, maintained under controlled environmental conditions, were mated and the day sperm were found in the vagina was designated as day one of pregnancy. L-10503 and L-11204 in a vehicle of sesame oil containing 30% benzyl benzoate was administered on day 9, 13 or 15 of pregnancy at doses of 50 or 100 mg/Kg in single subcutaneous injections. A total of 8–30 rats per group were employed. Animals were sacrificed by decapitation at various days of pregnancy. Their placenta, uteri, lungs and kidneys and fetal lungs or whole fetuses from these animals were rapidly dissected, blotted on filter paper to remove excess blood and fluid and immediately frozen on dry ice maintained at $-80°C$ until assayed.

Chemicals. Radioactive prostaglandins [5,6-^{3}H]-PGE_1 (59 Ci/mmol) and [9-^{3}H]-$PGF_{2\alpha}$ (15 Ci/mmol) were purchased from The Radiochemical Centre, Amersham, England. The radiochemical purity of these compounds were ascertained in our laboratory and found to be >95% pure. [5,6,7,8,9,11,12,14,15-^{3}H]-arachidonic acid (80 Ci/mmol) was purchased from New England Nuclear Corp. and then subjected to further purification according to the method of Flower *et al.*[14]. Non-radioactive PGE_1 was purchased from Chinoin, Budapest, Hungary, and $PGF_{2\alpha}$ was provided to us by Dr. John Pike of the Upjohn Co., Kalamazoo, MI.

Biochemical Procedures. In vitro metabolism and synthesis of prostaglandins were determined according to the method previously described [9]. As in the studies of the *in vivo* metabolism of prostaglandins, animals under pentobarbital anaesthesia (50 mg/Kg i.p.) were injected intravenously with radioactive PGE_1 or $PGF_{2\alpha}$ (2 μC/animal; 0.18 Ci/mmole). The animals were sacrificed 10 seconds later and blood was collected either by decapitation or by sampling from the abdominal aorta. Plasma from each sample was acidified with 0.3 ml of 0.5 M citric acid and extracted with 5 ml ethyl acetate (2 ×). The combined organic phases, evaporated under nitrogen flux, were processed according to the method previously described [9]. Histamine assays were performed according to the method of Shore *et al.*[15] as modified by Burkhalter[16]. Histaminase activity was determined radiometrically using the method described by Snyder[17] with [^{14}C]-putrescine as the substrate with the exception that 30 mM aminoguanidine hydrogencarbonate was used to stop the enzymatic reaction. The protein concentrations were determined by the method of Lowry *et al.*[18]. Four to 15 pools of tissues, each pool containing tissues from 2–4 animals, were used for each data point and the data were statistically evaluated using an analysis of variance. A Dunnett's "*t*" test was employed when a comparison between groups was desired.

RESULTS

Figure 2 illustrates the changes in prostaglandin synthesis and metabolism in rat placental tissue. Synthesis of $PGF_{2\alpha}$ and PGE_2 is low during most of gestation with the exception of a sharp increase in activity on day 11 of pregnancy and a return to a low level of activity on day 16. On day 11 the synthesis of PGE_2 increases to a level of six times greater than that of $PGF_{2\alpha}$. Metabolism also increases on day 11 but returns to a lower level on day 14 and then increases again on day 16 and remains at this higher level for the remainder of pregnancy. PGE_1 metabolism was approximately eight times as high as $PGF_{2\alpha}$ metabolism throughout pregnancy.

Figure 3 demonstrates the results obtained on uterine prostaglandin synthesis and metabolism during pregnancy. Prostaglandin synthesis is low until day 16 of pregnancy when it starts to increase, reaching its highest level on the day of parturition. It is interesting that even though the same conditions of tissue homogenate incubation for the uterus and placenta was used, the predominant product for the uterus was $PGF_{2\alpha}$ and for the placenta it was PGE_2. Uterine prostaglandin metabolism in comparison to that of the placenta was relatively low throughout gestation. The prostaglandin metabolism in the uterus rose only slightly on days 16 and 18.

Administration of L-10503 at 100 mg/Kg or L-11204 at 50 or 100 mg/Kg on day 9 of pregnancy (Exper. 1) reduced $PGF_{2\alpha}$ metabolism in the placenta (Fig. 4). L-10503 was effective at 24, 48 and 72 h post treatment in significantly reducing the metabolism of

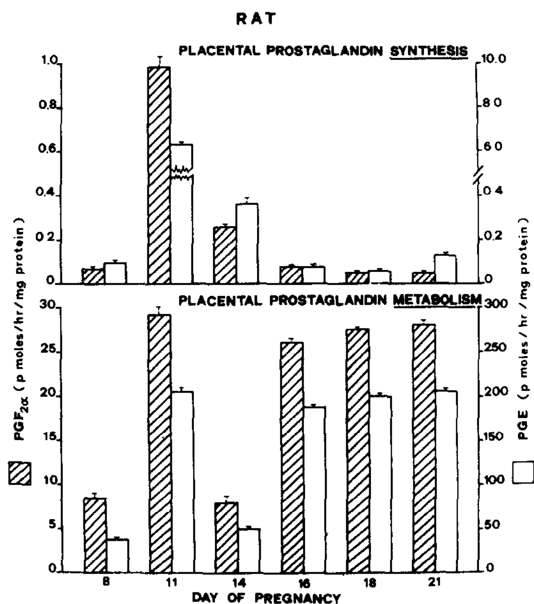

Fig. 2. Effect of day of gestation on prostaglandin synthesis and metabolism in homogenates of rat placenta. The S.E. of the mean is indicated by the vertical bar in this and other figures.

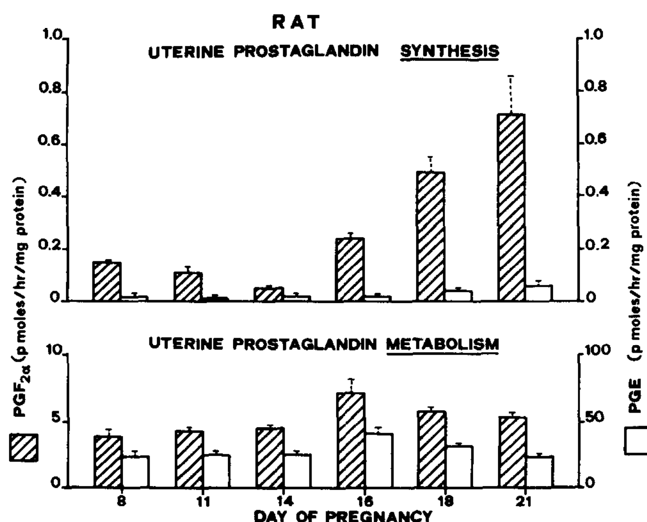

Fig. 3. Effect of day of gestation on prostaglandin synthesis and metabolism in homogenates in rat uteri.

prostaglandin whereas L-11204 did not alter this activity until 72 h post injection. L-10503 administered on day 13 of pregnancy (Exper. 2) did not affect prostaglandin metabolism, determined on day 16. In another study (Exper. 3) both compounds were administered on day 15 and no alteration of placenta prostaglandin metabolism was found at 24, 48 or 72 h post treatment. Similar determinations were made with uterine tissue (Fig. 5). Injection of L-10503 on day 9 of gestation produced a significant reduction in $PGF_{2\alpha}$ metabolism on days 10, 11 and 12 whereas injection of L-11204 on day 9 produced a significant effect only at 48 h post treatment and normal metabolism returned 24 h later.

$PGF_{2\alpha}$ metabolism in the lungs of pregnant rats was also inhibited by these compounds (Fig. 6). Treatment with L-10503 on day 9 of pregnancy produced a marked inhibition 24 h later and this effect was sustained over the 3-day sampling period (Exper. 1). Treatment with L-11204 on day 9 produced its effect on prostaglandin metabolism 48 h after injection and this effect was still present for the entire observation period of 10 days. In contrast to the results obtained with the reproductive tissues, injection of the compounds on days 13 or 15 of pregnancy (Exper. 2 and 3) significantly reduced prostaglandin metabolism. The effect of L-11204 treatment on lung tissue was also seen in the fetal lung (Table 1). When this com-

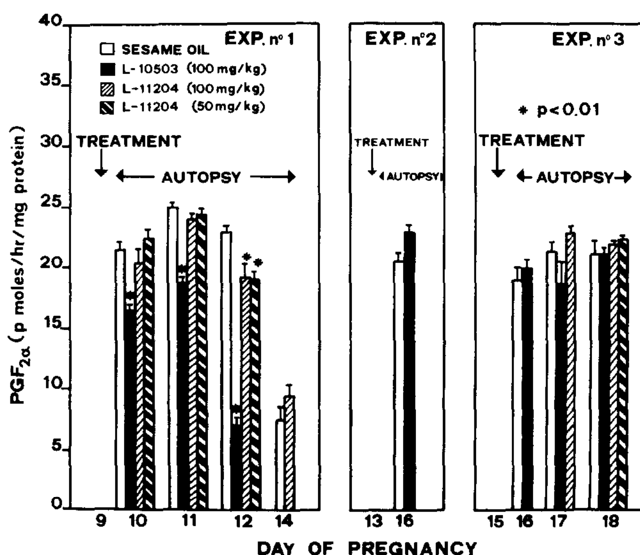

Fig. 4. Effect of L-10503 and L-11204 treatment on various days of pregnancy on $PGF_{2\alpha}$ metabolism in rat placenta.

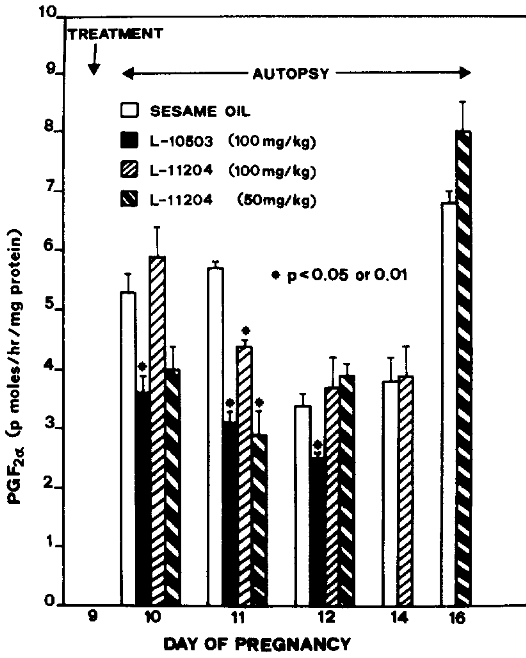

Fig. 5. Effect of L-10503 and L-11204 treatment on day 9 of pregnancy on $PGF_{2\alpha}$ metabolism in the rat uterus.

pound was injected into pregnant rats on day 15 of gestation, there was a 40% reduction in $PGF_{2\alpha}$ metabolism on days 19 and 21 of gestation.

In contrast to the results obtained with lung or reproductive tissues, treatment with L-10503 and L-11204 on day 9 of pregnancy did not alter kidney prostaglandin metabolism. Figure 7 illustrates this lack of effect on PGE_1 metabolism and similar negative results were obtained with $PGF_{2\alpha}$ metabolism.

All of the above studies employed a substrate concentration of labeled prostaglandin of 0.1 μM. It is possible that the results reflect isotopic dilution due to the influence of the pharmacological agents on the level of endogenous prostaglandin. An experiment was performed to exclude this possibility from the interpretation of the results. The rat lung was chosen to illustrate the results obtained (Fig. 8), although similar results were obtained with several tissues. The rate of prostaglandin metabolism when various concentration of PGE_1 was used as a substrate was measured. This rate was directly related to the substrate concentration. Therefore a concentration of 1000 times that previously used (0.1 mM *vs* 0.1 μM) was selected for the study of the effect of L-11204 treatment on day 9 of pregnancy. Table 2 shows that this treatment resulted in an 80% inhibition of the metabolism of PGE_1 demonstrating that the effect on this end point was real.

Since the studies described above were conducted in broken cell preparations where the compounds were administered to animals or added to an *in vitro* system, it was of interest to find out if this effect of the abortifacient was limited to the pregnant animal and if the compounds would alter the level of administered prostaglandin recoverable in the blood. Therefore, L-10503 was subcutaneously administered in a single dose of 100 mg/Kg to male and non-pregnant female rats and 24 h later [^{3}H]-PGE_1 was given i.v. and blood was sampled 10 seconds later. The results of this study are shown in Table 3. In both sexes there was a significant increase in recoverable PGE_1. Difference in sampling technique for blood collection did not influence results. A similar study using female rats to study the effect of various doses of L-10503 on [^{3}H]-$PGF_{2\alpha}$ metabolism was performed (Table 4). In this study decapitation was used as the method for blood collection. Doses as low as 10 mg/Kg of the compound significantly increased the percent of

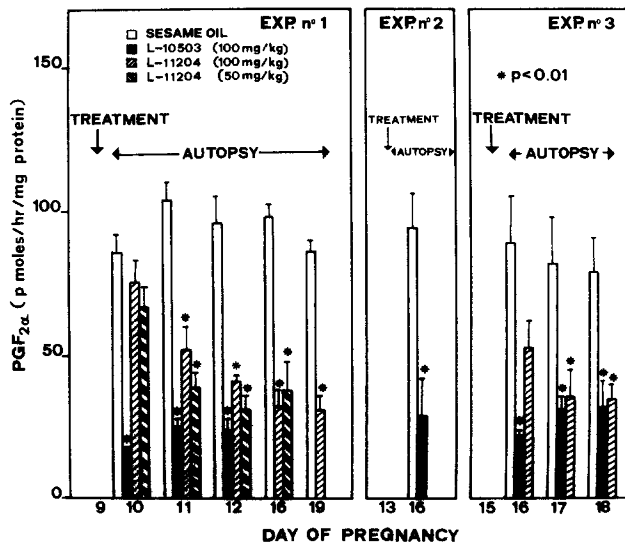

Fig. 6. Effect of L-10503 and L-11204 treatment on various days of pregnancy on $PGF_{2\alpha}$ metabolism in the rat lung.

Table 1. Effect of L-11204 treatment (100 mg/Kg, s.c.) on gestation day 15 on $PGF_{2\alpha}$ metabolism in rat fetal lung

Pregnancy day of autopsy	pmol/h/mg protein (mean ± S.E.)	
	Controls	L-11204
19	27.9 ± 0.5	18.8 ± 2.0*
21	51.6 ± 2.6	31.6 ± 1.7*

* $P < 0.01$ *vs* controls.

recovered $[^3H]$-$PGF_{2\alpha}$ in the plasma. There was no dose response and all of the doses up to 100 mg/Kg produced a doubling of recoverable prostaglandin. This probably indicates that we had reached a plateau for inhibitory activity and that even lower doses would be effective in preventing prostaglandin catabolism.

The effect of the pregnancy termination compounds on histamine and histaminase in reproductive tissues of the pregnant rat were also investigated. Table 5 shows the results of L-11204 administration on day 9 of gestation on placental histaminase on pregnancy days 10, 11 and 12. Histaminase activity in control rat placenta increased by 300% and 600% on days 11 and 12 compared to that of day 10. L-11204 at either dose employed did not alter this activity in day 10 placenta, however, there was a significant increase in this activity on days 11 and 12 following administration of the compound.

L-11204 at 100 mg/Kg but not at 50 mg/Kg also increased the histamine concentration in the uterus and placenta on day 12 of pregnancy in rats treated with the compound on day 9 (Table 6). The histamine contents of these tissues were approximately 137% that of the non-treated animals. Histamine content of the fetus on day 15 of gestation also was increased when the mother was administered 50 or 100 mg/Kg of L-11204. (Table 7). In the case of the fetus both doses produced maximal effects.

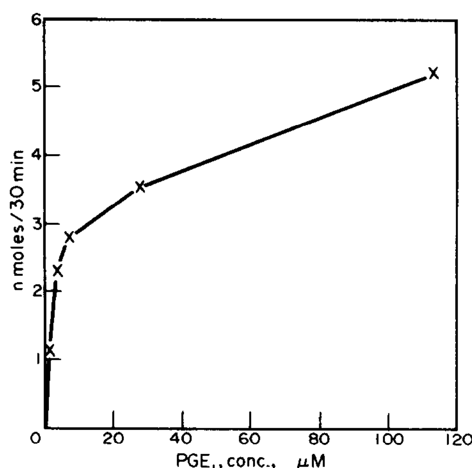

Fig. 8. Effect of variations in substrate concentration on the rate of PGE_1 metabolism by rat lung homogenate.

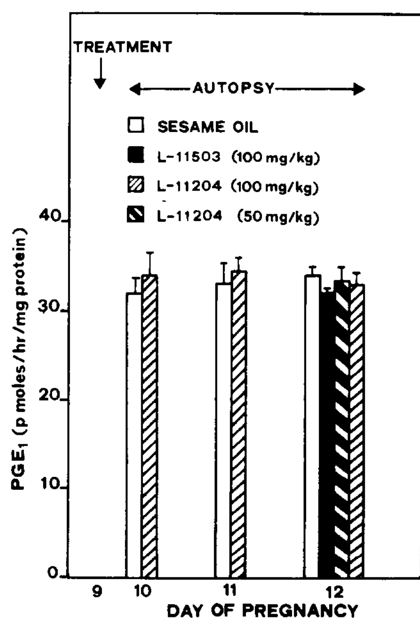

Fig. 7. Effect of L-10503 and L-11204 treatment on day 9 of pregnancy on PGE_1 metabolism in the rat kidney.

DISCUSSION

The level of prostaglandin synthetic and metabolic activity in the placenta and uterus changes during the course of pregnancy in the rat [9, 19]. Prostaglandin synthesis in the placenta is characterized by a sharp peak around day 11 of pregnancy. Progesterone concentration in the placenta also peaks at this time [20] but unlike prostaglandin synthesis which is at a high level in the placenta and at a low level in the uterus, progesterone concentration is high in both these tissues at that time. Also whereas progesterone concentration is at a low and declining level in the uterus during the last third of pregnancy, the synthesis of $PGF_{2\alpha}$ increases during that stage of gestation reaching its highest level just before parturition. Metabolism of prostaglandin in the placenta is high on day 11 also but unlike prostaglandin synthesis, this activity is also high during the last third of pregnancy.

The ratio of PGE and $PGF_{2\alpha}$ in the placenta is different from that in the uterus, probably reflecting physiological requirements. The relative quantities of each of these classes of prostaglandins is dependent upon the enzyme concentration in the tissue at each

Table 2. Effect of L-11204 (50 mg/Kg, s.c.) treatment on gestation day 9 on PGE_1 metabolism in rat lung*

	pmol/h/mg protein (mean ± S.E.)
Vehicle control	2753 ± 450
L-11204	580 ± 74†

* Autopsy on day 12 of pregnancy, incubation medium contained 0.11 mM PGE_1.
† $P < 0.01$ *vs* vehicle control.

Table 3. Metabolism of $[^3H]$-PGE_1 (2 μCi, 4 μg/animal, i.v.) administered 24 h after L-10503 in rats

L-10503 (mg/Kg, s.c.)	% of plasma radioactivity recovered as $[^3H]$-PGE_1	Sex	Blood source
—	11.4	Female	Decapitation 10 sec after $[^3H]$-PGE_1
100	32.9*		
—	7.4	Male	Abdominal aorta 10 sec after $[^3H]$-PGE_1
100	31.2*		

* $P < 0.01$ *vs* vehicle control.

Table 4. Metabolism of $[^3H]$-$PGF_{2\alpha}$ (2 μCi, 4 μg/animal, i.v.) administered 24 h after L-10503 in female rats

L-10503 (mg/Kg, s.c.)	% of plasma radioactivity recovered as $[^3H]$-$PGF_{2\alpha}$*
—	28.8
10	54.0†
50	59.0‡
100	58.7‡

* Blood obtained by decapitation 10 sec after $[^3H]$-$PGF_{2\alpha}$.
† $P < 0.05$ *vs* vehicle control.
‡ $P < 0.01$ *vs* vehicle control.

Table 5. Effect of L-11204 treatment on gestation day 9 on rat placental histaminase activity

L-11204 (mg/Kg, s.c.)	Pregnancy day 10	Pregnancy day 11	Pregnancy day 12
—	94 ± 5*	310 ± 3	626 ± 42
50	100 ± 17	507 ± 11†	1530 ± 61†
100	103 ± 13	741 ± 30†	1092 ± 154†

* c.p.m./mg protein, mean ± S.E. (total radioactivity extracted with toluene after 10 min incubation of tissue homogenate with $[^{14}C]$-putrescine.)
† $P < 0.01$ *vs* vehicle control.

Table 6. Effect of L-11204 treatment on gestation day 9 on rat uterine and placental histamine content*

L-11204 (mg/Kg, s.c.)	Histamine content (μg/g tissue, mean ± S.E.) Uterus	Placenta
—	2.24 ± 0.05	1.08 ± 0.09
50	2.04 ± 0.34	1.20 ± 0.05
100	3.07 ± 0.22†	1.47 ± 0.09†

* Determined on 12th day of pregnancy.
† $P < 0.01$ *vs* vehicle control.

Table 7. Effect of L-11204 treatment on gestation day 9 on histamine content of the rat fetus obtained on day 15 of pregnancy

L-11204 (mg/Kg, s.c.)	Histamine content (mean ± S.E.)		
	μg/g tissue	ng/mg protein	ng/whole fetus
—	2.46 ± 0.12	43.2 ± 2.0	392 ± 21
50	4.31 ± 0.46†	72.7 ± 7.6*	571 ± 26†
100	4.31 ± 0.45†	74.9 ± 9.8*	570 ± 53†

* $P < 0.05$ *vs* sesame oil.
† $P < 0.01$ *vs* sesame oil.

stage of gestation. Elevation of progesterone concentration and prostaglandin synthesis and metabolism a few days after nidation may indicate a period of vulnerability and of a physiological protective mechanism. Alteration of either the progesterone level or prostaglandin balance at that time may be detrimental to the continuation of the pregnancy.

Placental prostaglandin metabolism and synthesis and progesterone are not the only factors which change with the stage of pregnancy. It has been shown by Kameswaren, Pennefather and West[21] that histamine metabolism changes during gestation. They found that histaminase activity in the rat uterus and placenta rises sharply from day 10 to a peak on day 13 of pregnancy. Our data on rat placenta confirms this rapid increase in histaminase and is coincident with the rise in prostaglandin synthesis during this period of time.

In toxemia of pregnancy there is an alteration in the metabolism of placental prostaglandin [22] and histamine [13]. These parameters were examined when the pregnancy terminating compound, L-11204 was administered to the rat a few days after blastocyst implantation in the uterus. This treatment inhibited prostaglandin metabolism in placenta and uterus. Previously it was shown that the compound L-10503 inhibited placental and uterine prostaglandin metabolism without inhibiting synthesis of prostaglandin [11, 12]. L-11204 also induced an elevation of placental histaminase and increased uterine and placental histamine. Moreover such treatment also increased fetal histamine.

Reproductive tissues, however are not the only tissue affected by treatment with these pregnancy terminating compounds. Lung tissue prostaglandin metabolism was sharply decreased by treatment and this effect was prolonged for many days post treatment. However, no visible signs of respiratory distress was evident. A possible explanation of this lack of effect on pulmonary function may be that both $PGF_{2\alpha}$ and PGE_1 metabolism were inhibited therefore preventing an imbalance in the ratio of the dilation to constriction effects of the two classes of prostaglandins. The effect on prostaglandin metabolism was also seen in fetal lung tissue, probably indicating that the compound or a metabolite crossed the placental barrier. In contrast there was no effect at this endpoint in kidney tissue, demonstrating tissue specificity for this activity.

An explanation for the apparent difference in the duration of the effect of the compounds on reproductive tissues versus that of the lungs may be that pregnancy-induced changes in hormonal balance may control prostaglandin metabolism in the reproductive organs. Also the rapidly proliferating utero-placental unit may be rapidly diluting the compound available for enzyme interaction whereas the cell vol. of the lungs are in a relatively steady state.

Inhibition of metabolism of prostaglandins by these antifertility agents is not dependent upon the state of pregnancy or the sex of the animal since L-10503 given to male and non-pregnant female rats caused a significant increase in recoverable [^{3}H]-PGE_1 and [^{3}H]-$PGF_{2\alpha}$ in the plasma. Moreover, the dosage required for this effect was only a fraction of that required to abort a pregnant rat [10, 11]. However, it is a possibility that the prolonged elevation of plasma prostaglandins during the sensitive post ova implantation stage of pregnancy is involved in the mechanism of pregnancy termination activity of the compounds.

The effect on prostaglandin metabolism and histamine is not due to an effect on protein synthesis since *in vivo* studies using a labeled amino acid showed that these anti-fertility compounds did not alter protein synthesis in the lung or in the uteroplacental fetus complex [23]. The *in vivo* studies described here indicate that the compounds do not exert their effect on prostaglandin metabolism by inhibiting transport of prostaglandin into the cell but rather there is a direct inhibition of the enzyme systems involved. The system inhibited is the 15-hydroxy-prostaglandin dehydrogenase since in each experiment the metabolites (15-keto prostaglandin and 13, 14-dihydro-15-keto prostaglandin) as well as the non-metabolized prostaglandin were identified.

These studies suggest that the integrity of the utero-placental fetus unit during the post ova implantation period until mid-pregnancy is dependent upon a balance of the prostaglandins in each of the tissues. Alteration of this balance by interference with prostaglandin metabolism through the use of the compounds L-10503 and L-11204 could be a factor in the antifertility activity of these compounds. The change in the metabolism of prostaglandins may also be related to the effect of these compounds on histamine metabolism. It is tempting to speculate that the altered level and balance of prostaglandins and the

elevation in reproductive tissue histamine and histaminase change the state of hemodynamics to induce anoxia in these tissues and cause the breakdown of physiological functions leading to termination of the pregnancy.

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