Effects of Progesterone and Gossypol on Monoamine Oxidase Activity in Human Term Placental Explant

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Med., 1991, 163 (1), 39-45 — Human placental explant was cultured, using fresh
full term placenta delivered by elective cesarean section ($n=10$), and monoamine
oxidase (MAO) activity was measured in the tissue by the spectrophotometric
method. Placentae were obtained from patients without complications in preg-
nancy. Chorionic villi 2 g was incubated for 3 hr under the 95% air-5% CO₂
humidified condition at 37°C. When progesterone was added in the media (100-
500 µg/ml), MAO activities significantly decreased, compared with the control.
The gossypol (100 µg/ml) treated group showed no inhibitory or stimulatory
effects on MAO activities, compared with the control. However, progesterone
(100-500 µg/ml) and gossypol (100 µg/ml) treated group showed the same value of
MAO activities, compared with the control. Those results indicate that gossypol
might block progesterone action which is inhibitory against MAO activity. The
mechanism of pharmacological function of gossypol was discussed. ———
progesterone; gossypol; monoamine oxidase; placenta; culture

Human placenta develops at the 12th week of gestation as an organ which can
play a relatively complete role for the nutrition and maturation of fetus (Nesbitt
1974). Normal pregnancy is terminated after delivery of placenta following the
childbirth. Lots of enzymes, hormones, peptides, arachidonic acids and so on
produced in the placenta are indispensable during pregnancy (Contractor 1983;
Simon and Buster 1986). Some enzymes can be produced in vitro in the placenta
as time-dependent and hormone-dependent manners (Lewinsohn and Sandler

1982; Ishida et al. 1985). While steroid-converting enzymes or catecholamine-metabolizing enzymes abundantly exist in the placenta, placenta is mimic to liver (Nandakumaran et al. 1983; Sherman 1983). Monoamine oxidase (MAO), a catecholamine metabolizing enzyme, exists in the outer membrane of the mitochondria and might protect the fetus from catecholamines produced by the mother (Hsu and Shih 1986). The placental explant study shows Ca antagonist such as verapamil inhibits MAO activity (Barnea et al. 1986). (BU)₂ AMP and theophylline can stimulate MAO activity in the in vitro monolayer culture of neuroblastoma (Nakano et al. 1985). It is interesting to know what kinds of drugs other than those have inhibitory or stimulatory effects on MAO in the placenta. In this study, fresh term placentae ($n=10$) were cultured. The effects of progesterone and gossypol for MAO activity were investigated in the placental explant.

MATERIALS AND METHODS

Term placentae were obtained from 10 patients on elective cesarean section prior to the onset of labor in the OSU Hospital (placental weight, 580 ± 80 (mean $\pm$ s.d.) g; birth weight, 3450 ± 120 g, Apgar score, 9.0 ± 0.2 ; gestational weeks, 38.5 ± 0.4). Patients had no complications in pregnancy. Placenta was immediately placed into sterile normal saline at 4°C with ice and transported to the laboratory. The following steps were performed at 4°C cold room, except for the mentioned. Placental villous tissues were dissected from chorionic membranes and deciduas. Villous tissues were rinsed several times with Dulbecco's Modified Eagle Medium (DMEM) to eliminate red blood cell, and were cut approximately into 1 mm³ explants by scissors. Under the sterile condition, placental villi (2 g) was placed in a 60 $\times$ 15 mm Falcon culture dish (Falcon Plastics, Oxnard, CA, USA). This was done in 3 different dish. Placental explant culture was maintained for 3 hr under a 95% air-5% CO₂ humidified incubator at 37°C. Then, they were transferred to the 50 ml centrifuge tubes and centrifuged by 300 $\times$ *g* for 5 min at 4°C. The supernatant was thrown away. Villous tissue was put in the 50 ml centrifuge tube with 0.01 M Tris-HCl buffer 20 ml with 0.25 M sucrose (pH 8.0), and homogenized by a Brinkmann homogenizer for 30 sec 3 times. The homogenate was centrifuged at 600 $\times$ *g* for 10 min, and the supernatant was assigned to be a mitochondria fraction (enzyme source). MAO was determined according to the Weissbach's method with a slight modification. The rate of disappearance of kynuramine was detected by a Beckman spectrophotometer (Weissbach et al. 1960). The aliquot of 0.3 ml of 0.1 M kynuramine dihydrobromide, 0.3 ml of 0.5 M phosphate buffer, enzyme solution (0.05, 0.1, 0.2 and 0.3 ml) and deionized distilled water was incubated for 10 min at 37°C in the cuvette. A blank cuvette as control was prepared with water. The absorbance at 360 m μ was detected after 10 min incubation. The protein concentration of the enzyme solution was measured by Biorad protein assay kits. MAO activity was determined in the explant cultured for 0, 1, 2, 3 and 4 hr (Table 1). All the groups except the 4 hr-cultured group showed no significant change of MAO activity among the groups. Therefore 3 hr incubation was designed in our study (Table 2). Group 1 was the control. Group 2 was in the media contained progesterone of 100 μ g/ml, and group 3 in the media contained progesterone of 500 μ g/ml, group 4 in the media with progesterone (100 μ g/ml) and gossypol (100 μ g/ml), group 5 in the media with progesterone (500 μ g/ml) and gossypol (100 μ g/ml), and group 6 in the media with gossypol of 100 μ g/ml. Progesterone and gossypol were purchased from Sigma (St. Louis, MO, USA), dissolved in 100% ethanol, and sterilized by 0.2 μ m syringe filters (Gelman Sciences, Ann Arbor, MO, USA) prior to the experiment. The placental explants were cultured in the presence of progesterone and/or gossypol.

TABLE 1. The time dependent effect on MAO activity and on protein concentration of cultured placental explant

Group name	Sample number	Components of cultured media			Incubated hours	MAO activity n mol/mg of protein/min (mean $\pm$ s.d.)	Protein concentration mg/ml (mean $\pm$ s.d.)
		DMEM volume (ml)	100% ethanol volume (ml)				
A	10	9.98	0.02		0	1.4865 (0.0500)	5.4172 (0.2636)
B	10	9.98	0.02		1	1.4533 (0.0850)	2.6540 (0.0980)
C	10	9.98	0.02		2	1.4025 (0.1250)	2.7250 (0.1012)
D	10	9.98	0.02		3	1.4496 (0.0455)	2.7301 (0.0876)
E	10	9.98	0.02		4	1.3016 (0.0350)	2.6854 (0.0785)

MAO activity and protein concentration were determined in the placental explant cultured for 0, 1, 2, 3 and 4 hr. All the groups except the 4 hr-cultured group showed no significant change of MAO activity between the groups. Group A (no incubation) had almost two times higher protein concentration than did the other groups, indicating the effect of incubation on the protein concentration.

TABLE 2. *The effects of progesterone and/or gossypol on MAO activities of 3 hr-cultured placental explants*

Group name	Sample number	Components of cultured media				MAO activity n mol/mg of protein/min (mean $\pm$ S.D.)	Protein concentration mg/ml (mean $\pm$ S.D.)
		DMEM volume (ml)	100% ethanol volume (ml)	Progesterone (20 mg/ml) in 100% ethanol volume (ml)	Gossypol (20 mg/ml) in 100% ethanol volume (ml)		
1	10	9.98	0.02	0	0	1.4496 (0.0455)	2.7301 (0.0876)
2	10	9.98	0.015	0.005	0	1.3044 (0.0863)	2.4094 (0.0542)
3	10	9.98	0.01	Progesterone (50 mg/ml) in 100% ethanol 0.01	0	1.3048 (0.1792)	2.5560 (0.2064)
4	10	9.98	0.01	Progesterone (20 mg/ml) in 100% ethanol 0.005	0.005	1.5436 (0.1662)	2.3810 (0.1950)
5	10	9.98	0.005	Progesterone (50 mg/ml) in 100% ethanol 0.01	0.005	1.4717 (0.0285)	2.5161 (0.0691)
6	10	9.98	0.015	0	0.005	1.4500 (0.0451)	2.5010 (0.0540)

The components of cultured media per one dish are shown. Progesterone significantly decreased MAO activities of groups 2 and 3, compared with those of group 1 (control group). However, MAO activities of group 4 and 5 with gossypol and progesterone were significantly recovered, compared with those of groups 2 and 3. Group 6 showed no significant difference of MAO activity compared with group 1. Group 1 had higher protein concentration than did the other groups, indicating the effect of incubation time on the protein concentration.

RESULTS

Effect of progesterone on MAO activity of cultured placental explant

MAO activities of groups 2 and 3 significantly decreased compared with those of group 1 for 3 hr incubation. However, MAO activities of groups 4 and 5 with gossypol and progesterone were significantly recovered, compared with those of groups 2 and 3 (Table 2). Effect of gossypol on MAO activity of cultured placental explant. Group 6 showed no significant difference of MAO activity, compared with that of group 1 (Table 2).

Effect of progesterone and/or gossypol on protein concentration of cultured placental explant

The supernatant of the homogenate of villous tissue was used for protein assay. The protein concentration of fresh samples (no incubated : group A) was significantly higher than that of the incubated group (group B, C, D and E) (Table 1). However, no significant difference was detected in the protein concentration among the incubated groups (Table 2).

DISCUSSION

In our preliminary study, MAO activity of placental explant remains to be stable during 3 hr incubation. Progesterone 100 or 500 $\mu\text{g/ml}$ suppressed MAO activity in the placental explant. In placental explant, progesterone in more than 5–20 $\mu\text{g/ml}$ suppresses production of hCG for as long as 144 hr (Wilson et al. 1980). In short period of incubation, progesterone of the higher dose than that in Wilson's study exerts an inhibitory effect on MAO activity of placental explant in the presented data. Progesterone and gossypol in the media prohibits the inhibitory effect of progesterone on MAO activity. Gossypol might block the function of progesterone for MAO activity, while gossypol alone (100 $\mu\text{g/ml}$) had no effect on MAO activity in the placental explant. Gossypol is a yellowish compound extracted from cotton seeds and has been used as a contraceptive agent for animals and human (Lin et al. 1985). Gossypol exerts an inhibitory effect of inorganic anion transport as a membrane active agent (Haspel et al. 1985 ; de Peyster et al. 1986), and an antimitochondrial effect (Robinson et al. 1986 ; Benz et al. 1987), where gossypol induces the swelling or vacuolization of mitochondria of the melanoma in the monolayer culture study (Hu et al. 1986). The presented data suggests that gossypol has a potential as a blocker of progesterone action which is inhibitory for MAO activity. The possible mechanism of gossypol action is considered to be as follows ; (1) inactivation of progesterone by gossypol. (2) inactivation of progesterone receptors on trophoblast by gossypol competing progesterone. While the pharmacologic effect and mechanism of action of gossypol still remain to be clarified, gossypol might block progesterone action.

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