Effect of hexoprenaline on uteroplacental blood flow in the pregnant rat

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The effect of β-adrenergic agonists on uteroplacental blood flow is controversial. Human studies, with the use of indirect methods to assess uteroplacental blood flow, show conflicting results. Animal studies in the near-term pregnant sheep model have the disadvantages that the sheep has a syndesmochorial placenta and that the uteroplacental vessels are thought to be maximally dilated near term. The effect of hexoprenaline, a new β_2-sympathomimetic drug, was assessed in the awake pregnant rat on day 14 of gestation by means of the radionuclide-labeled microsphere method. Hexoprenaline increased placental blood flow by 198% and distribution of cardiac output to the placentas by 229%. Renal blood flow was reduced by 24%. Saline solution administration produced no significant effects. (Am J Obstet Gynecol 1986;154:310-4.)

Key words: Uteroplacental blood flow, hexoprenaline, β-adrenergic agonists

The effect of β-adrenergic stimulants on uteroplacental blood flow in pregnancy remains controversial. Animal studies, mainly with the use of the near-term pregnant sheep model, have generally failed to show an increase in uteroplacental blood flow after administration of these drugs. However, this model has two main disadvantages: (1) The uteroplacental vessels of the near-term pregnant sheep are thought to be maximally dilated and therefore further increases would not be expected 1; (2) the sheep has a syndesmochorial placenta, unlike the rhesus monkey, rabbit, rat, and man, which have hemochorial placentas.

In the human, various techiques to measure uteroplacental blood flow have been used. However, these methods only indirectly reflect uteroplacental blood flow and have produced conflicting results. Decreased placental perfusion may result in intrauterine growth retardation and increased fetal morbidity and mortality.

The various β-adrenergic agonists differ as to their receptor selectivity, placental passage, and cardiovascular effects. Based on equivalent tocolytic dosages, hexoprenaline was found to have less effect on maternal heart rate than either fenoterol, ritodrine, or salbutamol.[2] Use of carbon 14–labeled hexoprenaline failed to show any significant placental passage in pregnant rabbits.[3] To our knowledge the effect of hexoprenaline on uteroplacental blood flow has not been previously assessed. The fully conscious rat model at day 14 of gestation (term being 21 days) was chosen for this study as it has a hemochorial placenta and uteroplacental blood flow increases markedly during the last week of pregnancy in this animal.

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Methods

Timed pregnant Sprague-Dawley rats were obtained from Zivic-Miller Laboratories, Inc. (Allison Park, Pennsylvania) and were housed individually in stainless steel wire-bottom cages. Experiments were conducted on 13 rats at day 14 of gestation.

An 8 cm segment of polyethylene catheter (0.28 mm inside diameter by 0.61 mm outside diameter) was in-

Table I. Maternal and fetal weights

Variable	*Hexoprenaline*	*Placebo*	*p Value*
Maternal weight (gm)	334.6 ± 32.1	306.8 ± 39.5	0.19
Fetal weight (gm)	2.34 ± .36	1.94 ± .44	0.28
Litter size	13.6 ± 1.9	12.2 ± 2.6	0.28

Values are mean ± SD.

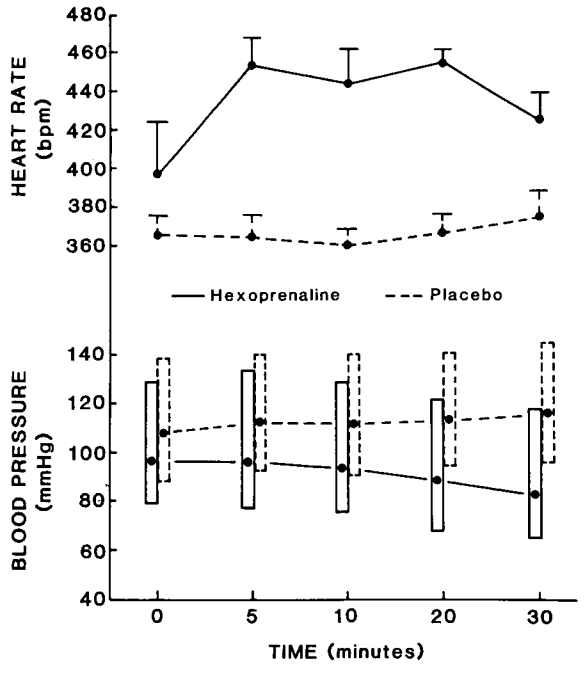

Fig. 1. Effects of hexoprenaline and saline solution on maternal heart rate and blood pressure. Heart rate is shown as mean ± SEM. Blood pressure is shown as systolic, mean, and diastolic pressures.

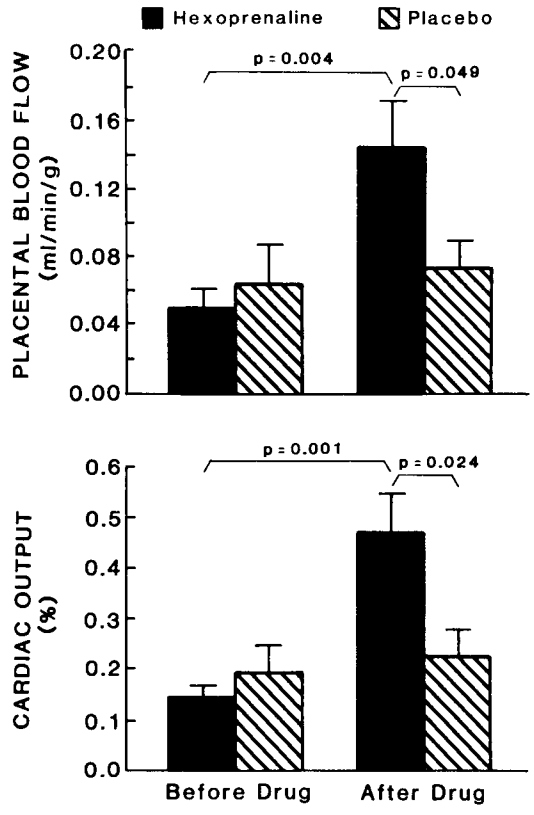

Fig. 2. Effect of hexoprenaline and placebo (saline solution) on maternal placental blood flow. *Top panel* expresses mean ± SEM flow as milliliters per minute per gram. *Lower panel* is the mean ± SEM percentage of total cardiac output distributed to the placentas.

serted into the end of a 30 cm segment of another polyethylene catheter (0.58 mm inside diameter by 0.97 mm outside diameter) and fused. With the animal under ether anesthesia, the small end of the catheter was inserted into the right carotid artery and advanced into the left ventricle. Placement of the catheter tip in the left ventricle was confirmed by the left ventricular pressure pulse tracing, and exact position was verified at autopsy. A similarly prepared catheter was inserted into the right external jugular vein and advanced 2 cm. A third catheter (0.58 mm inside diameter by 0.97 mm outside diameter) was inserted into the left femoral artery. The catheters were filled with heparinized saline solution (20 IU/ml) to prevent clotting and were then tunneled subcutaneously to a small hole in the skin at the back of the neck. From the exit point, the catheters were passed through a small metal spring to ensure that the animal would not chew on them. The animal was then placed in a holding cage and allowed a 3-hour recovery period. The femoral catheter was connected to a Statham P23Gb pressure transducer and arterial pressure and heart rate were recorded on a Gould 2200S recorder. The transducer was then disconnected and the catheter was attached to a constant withdrawal syringe pump (Harvard Apparatus, South Natick, Massachusetts), which was used to draw the arterial reference sample.

A suspension of approximately 100,000 microspheres labeled with either gadolinium 153 or tin 113 (New England Nuclear, Boston, Massachusetts), 15 ± 3 μm in diameter, were sealed in a 15 cm segment of silicone rubber tubing. The microspheres were suspended in physiologic saline solution with Tween-80 (0.01%) added to prevent aggregation. The microsphere-containing catheter was wound around a wooden dowel rod to a height of 1.5 cm, placed in a gamma counting vial, and counted in a Model 1185 gamma well counter (Nuclear-Chicago, Des Plaines, Illinois) at the appropriate photopeak for the isotope used. After counting, the ends of the catheter were clipped and one end was attached to the left ventricular catheter while the other was attached to a syringe containing 0.5 ml of saline solution. The femoral reference blood sample collection was initiated at the rate of 0.5 ml/min, and after verification that it was withdrawing smoothly, the microspheres were flushed into the left ventricle with the 0.5 ml of saline solution during a period of 20 seconds. Collection of the arterial reference sample was continued for 1 minute after the end of the microsphere injection to ensure that all microspheres in transit in the arterial blood were collected.

Table II. Blood flow to the various organs

Organ	*Before drug*			*After drug*		
	Hexoprenaline	*Saline solution*	*p Value*	*Hexoprenaline*	*Saline solution*	*p Value*
Placenta	0.141 ± 0.066	0.193 ± 0.128	0.373	0.465 ± 0.197	0.224 ± 0.118	0.024
Uterus	1.268 ± 0.390	1.255 ± 0.670	0.967	1.343 ± 0.311	1.184 ± 0.259	0.341
Ovary	1.878 ± 0.643	1.173 ± 0.694	0.084	1.612 ± 0.543	1.115 ± 0.679	0.170
Kidney	14.007 ± 2.592	15.684 ± 4.981	0.452	10.899 ± 3.520	14.542 ± 3.231	0.080
Vagina	0.173 ± 0.083	0.192 ± 0.045	0.628	0.152 ± 0.062	0.197 ± 0.086	0.302
Spleen	1.738 ± 0.820	1.865 ± 0.910	0.796	1.318 ± 0.626	2.007 ± 0.725	0.093
Heart	4.351 ± 2.209	5.216 ± 1.917	0.471	4.839 ± 2.372	5.743 ± 1.819	0.463
Lungs	2.229 ± 1.255	2.106 ± 0.869	0.843	2.262 ± 1.332	1.739 ± 0.952	0.440

Values are mean ± SD.

The arterial reference blood sample and syringe washings were transferred to a plastic gamma counting tube. The femoral catheter was flushed with saline solution and reattached to the pressure transducer, and heart rate and arterial blood pressure were recorded again. Blood pressure after microsphere injection did not differ significantly from that measured before microsphere injection, indicating that this method does not significantly alter the cardiovascular physiologic characteristics.

The venous catheter was connected to an infusion pump and the awake animal was given a bolus injection of saline solution or hexoprenaline (0.5 μg/kg = 0.25 ml/kg). The length of the entire catheter had previously been measured and appropriate adjustments made for the bolus volume. The bolus injection was followed immediately by a 30-minute infusion of saline solution or active drug, administered at a dosage of 0.1 μg/kg/min. Heart rate and blood pressure were monitored throughout the saline solution or drug administrations. Upon completion of the infusion, the alternate set of microspheres was injected and a second arterial reference sample drawn. Each animal was then killed with an overdose of sodium pentobarbital. The entire length of ventricular catheter and silicone rubber tubing was wound around the dowel rod again to a height of 1.5 cm, inserted into the gamma counting tube, and recounted in the gamma well counter. The counts per minute for the calculation of cardiac output were determined as the difference between the counts per minute of the microsphere catheter before injection and the counts per minute of the entire catheter after injection. Seven rats received hexoprenaline and six the saline solution.

After the rat was weighed, the uterus was removed and the fetuses and placentas were carefully detached from the uterine wall. The fetuses and placentas were separated from the amniotic fluid and membranes, gently blotted dry, and weighed separately; the placentas were placed in gamma counting tubes, three to four placentas per tube. The uterus was separated into right and left horns and placed in separate gamma counting tubes. The other organs and tissues were also placed in counting tubes to ensure that the levels of all tissues were kept below 1.5 cm to avoid loss of counting efficiency. The tissues and the arterial reference were counted in the gamma well counter, and the cardiac output and organ distribution of cardiac output were calculated as follows, where output and flow are measured in milliliters per minute and cpm is counts per minute:

$$\text{Cardiac output} = \frac{\text{cpm injected} \times \text{arterial reference flow rate}}{\text{cpm in arterial reference}}$$

$$\text{Organ blood flow (\% cardiac output)} = \frac{\text{cpm in organ}}{\text{cpm injected}} \times 100$$

$$\text{Organ blood flow} = \frac{\text{cpm in organ} \times \text{arterial reference flow rate}}{\text{cpm in arterial reference}}$$

In a preliminary experiment the whole animal was also cut up into pieces, which were placed in individual gamma counting vials (maximum tissue height in each vial = 1.5 cm) for gamma counting. The counts per minute injected in this case were calculated as the sum of the counts per minute of the whole animal. There was close agreement (<3% difference) in the counts per minute injected between this method and that of counting the counts per minute in the catheter before and after injection. Therefore, the differential counting of the catheter method was used in all subsequent experiments for convenience.

The statistical tests used were the Student *t* test for between-group comparisons and the paired *t* test to assess changes within the same groups.

Results

There were no significant differences in maternal weight, fetal weight, or litter size between the hexoprenaline and saline solution groups of rats (Table I). Cardiac output, heart rate, and blood pressure before

Organ	Mean difference			Hexoprenaline changes, paired t test (p value)
	Hexoprenaline	Saline solution	p Value	
Placenta	0.324 ± 0.144	0.032 ± 0.205	0.012	0.001
Uterus	0.076 ± 0.358	−0.071 ± 0.600	0.596	0.597
Ovary	−0.265 ± 0.718	−0.058 ± 0.555	0.578	0.366
Kidney	−3.107 ± 2.337	−1.142 ± 3.019	0.212	0.013
Vagina	−0.021 ± 0.068	0.005 ± 0.097	0.585	0.448
Spleen	−0.420 ± 0.776	0.142 ± 0.689	0.199	0.202
Heart	0.488 ± 1.474	0.527 ± 1.477	0.976	0.419
Lungs	0.033 ± 1.792	−0.367 ± 0.995	0.638	0.085

drug administration did not differ significantly between the two groups.

The mean (±SD) total cardiac output values after hexoprenaline and saline solution administration were 30.9 ± 6.89 and 30.1 ± 4.66 ml/min/100 gm, respectively. Hexoprenaline increased the mean heart rate and decreased the blood pressure (Fig. 1). Maternal placental blood flow was significantly increased (198%), from 0.141 ± 0.066 to 0.465 ± 0.197 ml/min/gm after hexoprenaline administration (Table II, Fig. 2). Blood flow to the kidneys significantly decreased (24%), from 14.0 ± 2.6 to 10.9 ± 3.5 ml/min/gm after hexoprenaline (Table III). The blood flows to the other organs were not affected by hexoprenaline. Administration of saline solution did not produce significant changes in the blood flows to any of the organs.

Comment

Compared with saline solution, hexoprenaline significantly increased blood flow to the placentas of pregnant rats on day 14 of gestation by increasing the fraction of cardiac output distributed to the placentas. This suggests vasodilation of the uteroplacental vasculature. In another study, with the diet-restricted pregnant rat model, long-term oral administration of hexoprenaline from day 5 of gestation resulted in a significant increase in placental blood flow measured on day 21 of gestation.[4]

In pregnancy α-adrenergic stimulation produces vasoconstriction and decreased blood flow in the uteroplacental circulation.[5] The vasculature of the nonpregnant uterus responds to β-adrenergic stimulation with vasodilation.[6] In pregnancy, however, the results of studies on β-adrenergic agonists are confusing and seem to depend on the specific drug, the species studied, and the experimental methodology. In near-term pregnant ewes, ritodrine, isoxsuprine, salbutamol, and terbutaline caused an increase in uterine vascular resistance and a decrease in uteroplacental blood flow.[7-9] Isoxsuprine had a greater effect than salbutamol or terbutaline. Fenoterol, on the other hand, caused a small reduction in uterine vascular resistance and an 11% increase in uterine blood flow.[9] In radioangiographic studies in near-term anaesthetized pregnant rhesus monkeys, Wallenburg et al.[10] found an increase in placental blood flow after administration of metaproterenol.

Studies in humans are encumbered by the necessity of having to use various techniques that may only indirectly reflect uteroplacental blood flow. Use of indium In 113m and gamma counting showed that fenoterol increased uteroplacental blood flow in the laboring patient[11] and that salbutamol decreased blood flow in the absence of uterine contractions.[12] With the use of xenon 133, fenoterol and isoxsuprine were shown to increase myometrial blood flow in nonlaboring women, but the intervillous blood flow remained unchanged.[13] With the use of a thermistor probe in the anterior lip of the cervix, ritodrine produced no change in blood flow in normal pregnancies, but in pregnancies with hypertension and intrauterine growth retardation, blood flow was significantly increased when compared with that after placebo.[14] Studies in the isolated human placenta showed that terbutaline did not change basal vascular resistance, but when the placental vessels were constricted with angiotensin, terbutaline produced a decrease in vascular resistance.[15]

Our finding that renal blood flow is significantly reduced after an intravenous infusion of hexoprenaline agrees with the results of Kleinman et al.,[16] who demonstrated a 70% reduction in fractional distribution of cardiac output to the kidney after an infusion of ritodrine in the pregnant ewe. These authors observed that the decrease in renal plasma flow was most likely due to active vasoconstriction, which may be mediated through an increase in renin-angiotensin levels. Available information appears to implicate circulatory overload as the primary factor in the cause of pulmonary edema produced by β-adrenergic receptor stimulants.

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Effect of long-term administration of β_2-sympathomimetic drug in the diet-restricted pregnant rat model

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To assess whether the maternal-fetal balance could be altered in favor of the fetus during malnutrition by increasing uteroplacental blood flow, 0.5 mg of hexoprenaline per day was added to the diet of one group of diet-restricted rats, while another group served as controls. The radionuclide-labeled microsphere method was used to determine blood flow to the maternal placentas and other organs. Maternal carcass weight but not fetal or placental weights were increased in the hexoprenaline-fed rats. Blood flow to the ileum, jejunum, hepatic artery, kidneys, and placentas was significantly greater in the hexoprenaline group compared with those rats fed the restricted diet alone. Although the placental blood flow was increased in the hexoprenaline-fed rats, the supply of nutrients remained restricted, and in the mother the inherent maternal-fetal balance was maintained by an increase in the blood flow to the liver and small intestine. (Am J Obstet Gynecol 1986;154:314-7.)

Key words: Dietary restriction, placental blood flow, hexoprenaline, β-sympathomimetic drug

Uteroplacental blood flow increases dramatically during pregnancy to support the nutritional demands of the rapidly growing fetus. This is made possible by an increase in maternal plasma volume and cardiac output, a decrease in vascular resistance, and an increase in the fractional distribution of cardiac output to the uterus.[1, 2]

A 50% dietary restriction in the rat from day 5 of pregnancy until term results in an absence of maternal weight gain beyond that of the conceptus.[2-4] At term, the diet-restricted rats were significantly smaller than the pregnant rats fed ad libitum, which was reflected

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