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Effects of Forskolin on Placental Vascular Resistance in Rabbits[1,2] (42759)

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Abstract. Forskolin is a direct stimulant of adenylate cyclase and increases cAMP production. It also acts as a vasodilator. To study the effect of forskolin infusion on rabbit maternal vascular resistance, we instrumented 11 pregnant rabbits with catheters in the left ventricle, jugular vein, and left and right femoral arteries. After a 2-day recovery period, one of two protocols was performed. In the control period of the first protocol ($N = 6$), 50% ethanol in saline was infused at 0.103 ml · min^{-1} for 5-min. Forskolin (10^{-3} M) in 50% ethanol was then infused for 5 min at 0.103 ml · min^{-1}. After each infusion period, regional blood flows were measured by microsphere injection. Data are expressed as means ± SEM. Blood pressure decreased from 81 ± 3 to 79 ± 3 mm Hg, ($P < 0.05$, $N = 10$) during forskolin infusion. Total placental resistance fell from 180.3 ± 10.7 to 133.8 ± 12.0 mm Hg · min · ml^{-1} per gram, $P < 0.05$. Cerebral, coronary, and renal vascular resistance fell significantly. During the second protocol ($N = 5$), angiotensin II (0.05 μg · min^{-1}) was infused for 5 min followed by the addition of forskolin (10^{-3} M at 0.103 ml · min^{-1}) to the infusate. Regional blood flows, vascular resistances and blood pressures were determined. Blood pressure fell from 99 ± 6 to 92 ± 7 mm Hg ($P < 0.05$) when forskolin was added to the infusate. Placental resistance fell from 202.5 ± 21.6 to 158.0 ± 29.0 mm Hg · min · ml^{-1} per gram ($P < 0.05$). While cerebral vascular resistance did not change, renal and coronary resistances fell in response to forskolin. This study demonstrates that forskolin is able to dilate rabbit placental vessels alone and in the presence of the vasoconstrictive agent angiotensin II.

Forskolin, an extract from the roots of the native Indian plant *Coleus forskohlii,* has been shown by several investigators to be a potent dilator of many vascular beds (1–3). There is also evidence which suggests that forskolin may have a tocolytic effect (4). In a recent study, Rankin and colleagues (5) reported that forskolin is able to vasodilate the maternal ovine placental vascular bed. The mechanism of this action appears to be via direct stimulation of the catalytic subunit of adenylate cyclase to produce increased levels of cAMP, causing smooth muscle relaxation (6). This mechanism does not require the presence of a membrane receptor.

To determine whether this action of forskolin is unique to the sheep, we infused forskolin intravenously and measured maternal placental resistance to blood flow in rabbits. In addition, we tested the ability of forskolin to exert a vasodilatory effect in the presence of the vasoconstrictor angiotensin II (7).

Materials and Methods. Eleven pregnant New Zealand white rabbits weighing between 3 and 5 kg were included in this study. Surgical procedures were performed on the 28th day of gestation in accord with our previously published protocol (8). Rabbits were anesthetized with an intravenous bolus of Nembutal sodium (19 mg/kg, Abbott). Catheters were inserted into the maternal jugular vein and left ventricle and the abdominal aorta via the right and left femoral arteries. The catheters were tunneled through the subcutaneous tissues and exteriorized on the nape of the neck where they were kept in a small pouch. Experimental procedures were conducted 2 days later. The study was divided into 2 protocols:

Protocol 1: Effects of forskolin infusion on regional vascular resistances. (a) Control. Infusion of vehicle (50% ethanol in saline) at 0.103 ml · min^{-1} for 5 min. *(b) Forskolin.* Infusion of 400 μg/ml forskolin (Calbiochem) in a 50% ethanol and saline solution at 0.103 ml · min^{-1} for 5 min.

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Protocol 2: Effects of forskolin infusion on preconstricted vascular beds. (a) Angiotensin II. Infusion of 0.50 $\mu g \cdot min^{-1}$ angiotensin II (Sigma) in 0.9% sodium chloride for 5 min. *(b) Angiotensin II/forskolin.* Infusion of angiotensin II (0.50 $\mu g \cdot min^{-1}$) and forskolin (400 $\mu g/ml$) at 0.103 $ml \cdot min^{-1}$ for 5 min.

All drugs were infused through the jugular vein catheter. Arterial blood pressure was monitored continuously throughout the experiment with use of a Statham P23dB transducer and Beckman R711 dynograph. The blood pressure signal was recorded as both pulsatile pressure and the electronic mean. Heart rate was calculated by counting the pulsatile pressure signal. Regional blood flows were measured at the end of each infusion period by injection of approximately 1.25 million radiolabeled microspheres of 15 μm diameter into the left ventricle. Right and left kidney flows were compared to determine whether adequate mixing of the microspheres was achieved. Reference blood samples were drawn at 2.06 $ml \cdot min^{-1}$ for 120 sec from the second femoral artery catheter beginning just prior to the time of microsphere injection. Blood samples were transferred to vials containing a 0.5-cm paraffin platform. Four isotopes were available for use and were chosen in random order: ^{125}I, ^{57}Co, ^{113}Sn, and ^{85}Sr. Each rabbit received a total of two isotopes.

Maternal arterial blood samples were analyzed (at 37°C) for PO_2, PCO_2 and pH. Total blood withdrawn during each experiment was approximately 10 ml. This loss occurred over a period of several hours and should not have affected experimental outcome.

Upon completion of the experiment, the doe was killed by injection of T-61 euthanasia solution (40 mg/kg, Hoechst). At necropsy, the uterus was removed and the placental tissue separated from uterine tissue. The kidneys, heart, and brain were also excised. Tissues were placed in wide mouth counting vials to a uniform height of 1 cm. Radioactivity of the blood and tissue samples was determined in a Nuclear Chicago 1185 sample changer with Norland 5400 multichannel analyzer. Blood flows were calculated from raw counts with use of the equation formulated by Makowski *et al.* (9).

Resistance was defined as average blood pressure during the 40-sec period following microsphere injection divided by blood flow per gram tissue weight, and is expressed as mm $Hg \cdot min \cdot ml^{-1}$ per gram. Data were analyzed by paired *t* test with $P \leq 0.05$ accepted as significant. All values are expressed as means ± SEM.

TABLE I. ARTERIAL BLOOD PRESSURES, REGIONAL VASCULAR RESISTANCES FOR PLACENTA, UTERUS, KIDNEY, BRAIN, AND HEART FOR PROTOCOLS 1 ($N = 6$) AND 2[a] (N = 5).

Protocol 1	Control	Forskolin	*P*
Placental resistance	180.3 ± 10.7	133.8 ± 12.0	0.025
Renal resistance	22.4 ± 2.2	17.8 ± 2.6	0.029
Cerebral resistance	124.3 ± 5.4	62.5 ± 2.6	0.0006
Coronary resistance	32.3 ± 3.8	17.9 ± 2.3	0.002
Blood pressure (mm Hg) ($N = 10$)	81 ± 3	79 ± 3	0.049
Protocol 2	**AII[a]**	**FORSK/AII[a]**	***P***
Placental resistance	202.5 ± 21.6	158.0 ± 29.0	0.021
Renal resistance	51.3 ± 5.4	33.5 ± 1.6	0.027
Cerebral resistance	164.6 ± 22.8	129.0 ± 17.7	NS[b]
Coronary resistance	40.3 ± 4.9	23.7 ± 3.4	0.047
Blood pressure (mm Hg)	99 ± 6	92 ± 7	0.012

Note. Resistance units are all mm $Hg \cdot min \cdot ml^{-1}$ per gram. Data are expressed as means ± SEM.

[a] AII, angiotensin II; AII/FORSK, angiotensin II and forskolin infusion.

[b] NS, not significant.

Results. The maternal arterial PO_2 was 105 ± 4 mm Hg, PCO_2 was 25.9 ± 2.8 mm Hg, and pH was 7.45 ± 0.01. Regional vascular resistances and blood pressures are presented in Table I.

Protocol 1. Effects of forskolin infusion on regional vascular resistances (N = 6). Forskolin infusion caused an increase in total placental blood flow from 0.45 ± 0.03 $ml \cdot min^{-1}$ per gram during control to 0.63 ± 0.08 $ml \cdot min^{-1}$ per gram ($P < 0.05$). Placental vascular resistance was 180.3 ± 10.7 mm $Hg \cdot min \cdot ml^{-1}$ per gram during control and fell to 133.8 ± 12.0 mm $Hg \cdot min \cdot ml^{-1}$ per gram in response to forskolin infusion ($P < 0.05$). This represents a fall of 26% from control. Resistance to renal blood flow fell 21% from control values during forskolin infusion. The cerebral vascular bed responded to forskolin infusion with a dramatic fall in resistance to 50% of its control value. Coro-

nary vascular resistance also fell 45% from the control value during forskolin infusion.

The change in arterial blood pressures was small in response to forskolin. Therefore, the influence of forskolin on blood pressure was studied in four additional animals. These animals received control and forskolin infusions after a 1-hr minimum recovery from the forskolin and angiotensin II infusions used in protocol 2. For these 10 animals, there was a small but statistically significant drop in blood pressure from 81 ± 3 mm Hg during control to 79 ± 3 mm Hg during forskolin infusion ($P < 0.05$, paired t test).

Protocol 2. Effects of forskolin infusion on preconstricted vascular beds (n = 5). As seen in Table I blood pressure declined significantly from 99 ± 6 mm Hg during angiotensin II infusion to 92 ± 7 mm Hg ($P < 0.05$) during simultaneous infusion of angiotensin II and forskolin. During angiotensin II infusion, placental blood flow per gram was 0.52 ± 0.03 ml · min^{-1} per gram; during angiotensin II and forskolin infusion, this rate was 0.65 ± 0.09 ml · min^{-1} per gram ($P = 0.055$). Placental vascular resistance was 202.5 ± 21.6 mm Hg · min · ml^{-1} per gram during infusion of angiotensin II only and fell to 158.0 ± 29.0 mm Hg · min · ml^{-1} per gram when forskolin was infused with angiotensin II ($P < 0.05$). Forskolin caused the renal vascular resistance to fall 35% from its initial value during angiotensin II infusion. Coronary resistance exhibited a significant decline of 41% during angiotensin II and forskolin infusion. The resistance of the brain blood vessels did not change to a significant degree.

Discussion. In this study, the effects of forskolin on the vascular resistance of normal and angiotensin II constricted placentas were studied in rabbits during late gestation. Maternal arterial pH and gas tension values indicate that the animals were healthy.

Forskolin (400 μg/ml) was infused at 0.103 ml · min^{-1} into rabbits weighing on average 4 kg. This infusion rate, 10 μg · min^{-1} · kg^{-1}, is similar to the rate which has previously been shown to increase cerebral, myocardial, and renal blood flows in nonpregnant anesthetized rabbits (2).

The choice of 15-μm-diameter radiolabeled microspheres was based on a recent study by Rankin *et al.* (10). This study compared placental resistance as measured by 15- and 25-μm microspheres and reported no significant difference between the two methods.

The results of this study indicate that forskolin causes a fall in placental, cerebral, myocardial, and renal resistance in normal conscious pregnant rabbits. It also shows that forskolin can dilate placental, renal, and myocardial vascular beds which have been constricted by angiotensin II.

The regulation of placental vascular resistance is of major physiologic concern since an adequate placental blood supply is a critical component of fetal survival. However, regulation of this vascular bed is not well understood. Greiss (11) demonstrated in sheep that the placental vascular bed does not autoregulate. This led to speculation that the placental vasculature is maximally vasodilated near the end of pregnancy. To test this hypothesis, Parisi *et al.* (12) constricted the ovine placental vasculature with angiotensin II and then infused the vasodilator Prostaglandin I_2 into the uterine artery. These investigators reported that there was no decrease in placental vascular resistance in response to Prostaglandin I_2 infusion. Landauer *et al.* (13) reported the same results in a similar experiment with adenosine.

Recently, Rankin *et al.* (5) demonstrated that the diterpene forskolin is able to decrease ovine placental vascular resistance when delivered locally to the uterine vascular bed in chronically catheterized ewes. The present study in rabbits indicates that the vasodilatory actions of forskolin are not specific to sheep and that similar results are observed when forskolin is delivered systemically instead of locally. These observations suggest that neither rabbit nor sheep placentas are maximally vasodilated. However, forskolin has been reported to cause myometrial relaxation (4). This action may play some role in the fall in placental vascular resistance. Finally, the vasodilation which occurred in the rabbit heart and kidneys in this study indicates that forskolin does not vasodilate the placenta at the expense of blood flow to other important maternal organs.

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