Urotensin-II Contributes to Pulmonary Vasoconstriction in a Perinatal Model of Persistent Pulmonary Hypertension of the Newborn Secondary to Meconium Aspiration Syndrome

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ABSTRACT: Meconium aspiration syndrome (MAS) disrupts perinatal decreases in pulmonary vascular resistance (PVR) and is the commonest cause of neonatal pulmonary hypertension. The contribution of the potent vasoactive agent urotensin-II (U-II), in the pathophysiology of this condition, is unknown. In a new perinatal model of MAS, we combined measurement of circulating U-II levels with U-II receptor blockade studies. Nineteen anesthetized lambs were instrumented then randomly allocated to the following groups: 1) control ($n = 5$), 2) control plus specific U-II receptor blockade with palosuran ($n = 5$), 3) tracheal instillation of meconium ($n = 5$), 4) meconium instillation plus palosuran ($n = 4$). Hemodynamics, PVR, and plasma U-II were measured for 6 h after delivery. After birth in controls, U-II increased ($p < 0.05$), and PVR fell ($p = 0.01$) and this fall was prevented by U-II receptor blockade. By contrast, meconium lambs displayed a greater rise in U-II levels ($p < 0.05$ versus control) with an increase in PVR ($p < 0.005$) that was attenuated by U-II receptor blockade ($p < 0.001$). These findings suggest that U-II normally acts as a pulmonary vasodilator after birth, but in the presence of MAS, it assumes a vasoconstrictor role. U-II receptor blockade also improves pulmonary hemodynamics in this model. (*Pediatr Res* 67: 150–157, 2010)

Meconium aspiration syndrome (MAS) is a severe neonatal respiratory disorder (1) and is the commonest cause of pulmonary hypertension of the newborn (PHN) (2). MAS disrupts the perinatal transition in the pulmonary circulation, so that, the usual fall in pulmonary artery pressure (P_{PA}) and pulmonary vascular resistance (PVR), and increase in pulmonary artery blood flow (Q_{PA}) are impeded. These abnormalities, in association with damage to lung parenchyma induced by aspirated meconium, may lead to critical hypoxemia and pulmonary hypertension (1–4).

Important knowledge regarding the pathophysiology of MAS has been obtained from animal models (1–4). However, a significant limitation of most existing models is that they have been performed in animals that are several days to weeks old and have already negotiated much of the perinatal transi-

tion. This limits the investigation of fundamental early events in the pathophysiology of MAS.

Animal studies (5,6), which are consistent with observations in humans (6), indicate that the fall in PVR after birth is dependent on the action of endothelium-related vasoregulators. An imbalance in these vasoregulators is a characteristic feature of PHN, with up-regulation of vasoconstrictor influences (6). The vasoconstrictor that has received greatest attention in MAS is endothelin-1 (ET-1). Circulating ET-1 levels parallel the rise in P_{PA} (7) and PVR (7) and antagonism of the ET receptor lowers these variables (2,8). However, as the hemodynamic abnormalities in MAS are not completely reversed by high doses of ET-receptor antagonists (2), it is clear that ET-1 is only one of a number of mediators in the pathophysiology of PHN in this setting.

A recently uncovered endothelial-derived vasoregulatory mediator is urotensin-II (U-II). U-II has potent vasodilator and vasoconstrictor actions and is synthesized in the pulmonary endothelium (9). Plasma and lung tissue homogenate levels of U-II (10,11) and expression of U-II by pulmonary arterial endothelial and smooth muscle cells (12) are elevated in different models of pulmonary hypertension. However, the potential contribution of U-II to the perinatal transitional circulation, and in particular, to the pathogenesis of PHN is unknown.

Accordingly, the aim of this study was to investigate the potential role of U-II in the neonatal pulmonary transitional circulation, in the absence and presence of MAS, by examining the changes in plasma levels of U-II and the effects of palosuran, a specific antagonist of the U-II receptor (13).

METHODS

Experiments were approved by The Animal Ethics Committee of Murdoch Children's Research Institute.

Abbreviations: ABG, arterial blood gas; CI, cardiac index; ET-1, endothelin-1; FiO_2 , fractional inspired oxygen; MAS, meconium aspiration syndrome; OI, oxygenation index; P_{Ao} , aortic pressure; P_{PA} , pulmonary artery pressure; PHN, pulmonary hypertension of the newborn; PVR, pulmonary vascular resistance; Q_{LPA} , left pulmonary artery blood flow; Q_{PA} , pulmonary artery blood flow; SVR, systemic vascular resistance; U-II, urotensin-II

Received June 3, 2009; accepted September 22, 2009.

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Supported by a postgraduate medical research scholarship from The National Health and Medical Research Council of Australia (to C.M.S.). This project was supported by a Grant-in-Aid from The National Heart Foundation of Australia.

Experimental preparation. Nineteen Border-Leicester cross ewes (gestation, 139 ± 2 d, term = 147 d) were anesthetized, intubated, and ventilated. O_2 saturation (SpO_2) was monitored continuously with a pulse-oximeter (Tyco Healthcare; CA) applied to the ear. Polyvinyl catheters were inserted into the external jugular vein and the common carotid artery. Ventilation was adjusted to maintain arterial PO_2 and PCO_2 within physiologic ranges. Body temperature was maintained at $38-40^\circ C$.

Fetal surgery. After delivery through a midline hysterotomy, the fetal head was placed in a warm saline-filled surgical glove. Polyvinyl catheters were advanced through the external jugular vein into the right atrium and through the common carotid artery into the ascending aorta. Arterial blood gas (ABG) samples were taken every 30 min.

After partial exteriorization of the fetus, the heart and great vessels were exposed through a left thoracotomy. A catheter was inserted into the left atrium for pressure measurement. A 10- to 14-mm diameter perivascular ultrasonic flow probe (model 10A-14A Transonic Systems; NY) was placed around the pulmonary trunk to measure Q_{PA} and, after closure of the DA, cardiac output. A 4- to 6-mm diameter ultrasonic flow probe (model 4S-6S; Transonic Systems) was placed around the left pulmonary artery to measure left lung flow (Q_{LPA}) and to calculate PVR. An additional catheter was inserted into the pulmonary trunk distal to the flow probe. The skin edges of the thoracic incision were closed with tissue forceps.

Study groups. Lambs were randomly allocated to one of four groups. The control group ($n = 5$) received routine monitoring and intensive care only. The palosuran group ($n = 5$) additionally received a continuous infusion of the specific U-II receptor antagonist, palosuran ($10 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$; Actelion Pharmaceuticals, Switzerland), commenced immediately after delivery and continued until the end of the experiment. The infusion rate was chosen according to previous studies (13) and pilot studies in newborn lambs, which demonstrated that this dose was well tolerated. The meconium group ($n = 5$) received 3 mL/kg of filtered 20% human meconium (1), instilled deeply into the trachea, and upper airways immediately after delivery. The meconium-palosuran group ($n = 4$) received both meconium and a palosuran infusion.

Delivery of lamb. After intubation of the fetal trachea, lung liquid was drained under gravity to enhance gas exchange, and the endotracheal tube was clamped to prevent lung aeration. The lamb was delivered onto a heated resuscitation table, and the umbilical cord was clamped and cut. After withdrawal of umbilical cord blood for replacement of volume in the lamb during subsequent blood sampling, the ewe was euthanized.

After delivery, control and palosuran animals received two manual chest inflations with a fractional inspired oxygen (FiO_2) of 1.0%, in the left lateral and then right lateral position. Meconium and meconium-palosuran animals also received two manual inflations, each preceded by instillation of half the total amount of meconium. Mechanical ventilation was commenced using a time-cycled, pressure-limited mode (Infant Star 500; Infrasonics, CA). For the control and palosuran groups, initial ventilator settings were a peak inspiratory pressure of 30 cm H_2O , a positive end expiratory pressure of 4 cm H_2O , a respiratory rate of 30/min, inspiratory time of 1.0 s, and FiO_2 of 0.3. Pilot studies indicated that a higher degree of ventilation was required early after meconium administration, so the initial ventilator settings in the meconium and meconium-palosuran groups were a peak inspiratory pressure of 35 cm H_2O , a positive end expiratory pressure of 6 cm H_2O , a respiratory rate of 35/min, inspiratory time of 0.8 s, and FiO_2 of 0.8.

Anesthesia was maintained with continuous infusions of ketamine ($4 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) and midazolam ($0.05 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$), and i.v. maintenance fluid was given. SpO_2 was monitored continuously with a pulse-oximeter applied to the cheek, and ABG analysis was performed frequently. FiO_2 was

adjusted to maintain arterial hemoglobin $O_2 > 95\%$ (14). Ventilation was adjusted to maintain PCO_2 at 40–45 mm Hg. Body temperature was maintained within physiologic limits ($38 \pm 0.1^\circ C$).

After a 15-min period of stabilization, initial baseline recording of hemodynamic variables and aortic blood gas analysis was performed. Subsequently, hemodynamic data were recorded, and aortic and pulmonary arterial blood samples were collected at 30 min intervals until 2 h and then hourly thereafter. As the DA normally closes very rapidly in lambs (15), it was occluded with a vascular clamp after the 1-h measurement, to remove the potentially confounding effect of any shunting on hemodynamics and blood gases.

After 6 h, lambs were euthanized and body and lung weights were recorded.

U-II and ET-1 assays. Pulmonary arterial blood samples were centrifuged at $4^\circ C$ and 3000 rpm for 10 min. Plasma was stored at $-80^\circ C$, and later analyzed for U-II and ET-1 by RIA (16,17).

Physiologic variables. Vascular pressures were measured via transducers (Transpac IV, Abbott Critical Care Systems, Ireland) referenced to atmospheric pressure at the level of the left atrium. Pressure signals were processed with a transducer amplifier (Transbridge TBM4M; World Precision Instruments, FL). Flows were measured with a flowmeter (model T206; Transonic Systems, NY). Physiologic signals were digitized at a rate of 1000 Hz and stored on computer using an analogue-to-digital converter (iNet-100B; GW Instruments, MA) interfaced with programmable acquisition and analysis software (Spike2; Cambridge Electronic Design, UK). A 48-Hz low-pass filter was applied during analysis to remove electrical interference.

ABG samples were measured with a blood gas analyser (Radiometer ABL 620; Denmark). PVR of the left lung was calculated as: $PVR = \text{mean } P_{PA} \text{ (mm Hg)} - \text{mean } P_{LA} \text{ (mm Hg)} / \text{mean } Q_{LPA} \text{ (mL/min)}$, where P_{LA} = left atrial pressure, and was normalized to 100 g of lung weight. Systemic vascular resistance (SVR) was calculated after closure of the ductus arteriosus as: $SVR = \text{mean } P_{Ao} \text{ (mm Hg)} - \text{mean } P_{RA} \text{ (mm Hg)} / \text{mean } Q_{PA} \text{ (mL/min)}$, where P_{Ao} = aortic pressure and P_{RA} = right atrial pressure, and normalized to body weight. Cardiac index (CI) was calculated using Q_{PA} after ductal closure (cardiac output) and indexed to body weight. Oxygenation index (OI) was calculated as $\text{mean airway pressure} \times FiO_2 \times 100 / Po_2$.

Statistical analysis. Data were analyzed with SigmaStat for Windows (v3.0; San Jose, CA), using one- and two-way ANOVA. *Posthoc* analysis to look for specific effects within group variables was performed by partitioning the sums of squares into individual degrees of freedom (18). *Posthoc* analysis of between group variables was performed using the Bonferroni correction. Results are expressed as mean $\pm$ SEM, with $p < 0.05$ considered statistically significant.

RESULTS

Baseline variables. Pulmonary hemodynamic variables, OI and plasma U-II and ET-1 levels were similar in all groups. Compared with controls, CI tended to be lower in the meconium group ($p = 0.08$) and was lower in the meconium-palosuran group ($p < 0.05$). Baseline SVR was lower in the palosuran group ($p < 0.05$; Table 1).

Effect of meconium aspiration. Between 15 and 360 min after birth, P_{PA} fell progressively in controls ($p < 0.001$) but

Table 1. Baseline variables in all study groups

Baseline measure	Control group	Palosuran group	Meconium group	Meconium-Palosuran group
P_{PA} (mm Hg)	49 (4)	41 (4)	44 (3)	49 (5)
Q_{LPA} ($\text{mL} \times \text{min}^{-1} \times 100 \text{ g}^{-1}$)	1201 (170)	1149 (225)	907 (61)	1065 (96)
Left lung PVR ($\text{mm Hg} \times \text{mL}^{-1} \times \text{min}^{-1} \times 100 \text{ g}^{-1}$)	0.037 (0.01)	0.031 (0.006)	0.039 (0.002)	0.036 (0.003)
Oxygenation index	10 (3)	7 (2)	11 (2)	13 (5)
P_{Ao} (mm Hg)	66 (3)	55 (6)	63 (3)	70 (7)
Cardiac Index ($\text{mL min}^{-1} \times \text{kg}^{-1}$)	198 (8)	183 (14)	153 (18)	136 (10)*
SVR ($\text{mm Hg mL}^{-1} \text{ min}^{-1} \times \text{kg}^{-1}$)	0.33 (0.01)	0.23 (0.03)*	0.33 (0.04)	0.4 (0.04)
U-II (pmol/L)	1.28 (0.1)	1.2 (0.1)	1.43 (0.12)	1.53 (0.05)
ET-1 (pmol/L)	8.1 (0.6)	14.2 (1.8)	11.7 (1.3)	10.5 (2.6)

Mean (SEM) hemodynamic variables, Oxygenation Index, U-II and ET-1 levels at first post-natal measure. The ductus arteriosus was occluded after the 60 min measurement. Note that Cardiac Index and SVR were first obtained at the 90 min time-point.

* $p < 0.05$ compared with control group.

Figure 1. Hemodynamic variables and OI in control (●, $n = 5$) and meconium (■, $n = 5$) groups. The dotted line at 1 h indicates the time of ductal occlusion. Results are expressed as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, † $p < 0.001$ (between groups).

was unchanged in the meconium group. Q_{LPA} fell in both controls and meconium animals ($p < 0.005$ for both) but remained higher in controls throughout the study period. Thus, although PVR fell progressively in controls ($p = 0.01$), it increased in the meconium group ($p < 0.005$). OI also fell progressively in controls ($p < 0.001$) but increased in the meconium group ($p < 0.01$) (Fig. 1).

P_{AO} was unchanged over the study period in controls, but fell in the meconium group ($p < 0.005$). As a result, the ratio between P_{PA} and P_{AO} fell in controls ($p < 0.001$) but increased in animals receiving meconium ($p < 0.01$) (Fig. 1).

After ductal closure, CI was maintained in both groups but was higher in controls compared with the meconium group. SVR fell in both ($p < 0.05$ for controls; $p < 0.001$ for meconium), although the fall was greater in the meconium group (Fig. 1).

Arterial pH, PO_2 , Pco_2 , Sao_2 , and Fio_2 did not change between 15 and 360 min after birth in controls; however, in meconium animals, PO_2 and Sao_2 fell significantly during this time. Over the whole observation period, pH and Sao_2 were significantly lower in meconium animals, compared with controls, whereas Pco_2 and Fio_2 were higher (Table 2).

During the study period, U-II levels increased in the control ($p < 0.05$) and meconium groups ($p < 0.001$) with the increase being greater in the latter. ET-1 levels were un-

changed in controls but increased in the meconium animals ($p < 0.025$) (Fig. 2).

Effects of palosuran in the absence of meconium. The fall in P_{PA} that occurred during the study period in controls was not altered by palosuran. However, palosuran resulted in an exaggerated fall in Q_{LPA} , so that the reduction in left lung PVR in controls was abolished by palosuran. Although the difference in PVR between control and palosuran groups was small, when analyzed in terms of percent change from baseline to 6 h, PVR in controls had fallen by 17% and in palosuran animals had increased by 24% ($p < 0.001$ for difference between groups).

OI initially increased in palosuran animals ($p < 0.01$), so that over the duration of the study, OI was greater in the palosuran group. P_{AO} was unchanged over the study period in controls but fell in the palosuran group ($p < 0.001$). As a result the ratio of P_{PA}/P_{AO} , which fell in controls increased in the palosuran group ($p < 0.001$). After ductal closure, CI was maintained in controls, but tended to fall in the palosuran group ($p = 0.06$), so that over the duration of the study, CI was lowered by palosuran. After ductal closure, SVR fell in controls ($p < 0.05$), and palosuran-treated animals ($p < 0.001$) but was lower in the palosuran group (Fig. 3).

In the palosuran group, pH, Pco_2 and Fio_2 did not change significantly between 15 and 360 min after birth, whereas PO_2 fell

Table 2. Arterial blood gases and inspired oxygen fraction in all study groups over time

											<i>P</i>			
											Within group	Control vs Pal	Control vs Mec	Mec vs Mec-Pal
Time (h)	0.25	0.5	1	1.5	2	3	4	5	6					
pH														
Control	7.30 (0.04)	7.37 (0.02)	7.40 (0.04)	7.38 (0.04)	7.35 (0.03)	7.35 (0.04)	7.39 (0.03)	7.34 (0.03)	7.32 (0.02)	N.S.				
Pal	7.30 (0.05)	7.30 (0.05)	7.30 (0.03)	7.28 (0.02)	7.29 (0.02)	7.31 (0.03)	7.31 (0.03)	7.28 (0.01)	7.27 (0.02)	N.S.	<0.001			
Mec	7.23 (0.04)	7.30 (0.02)	7.31 (0.03)	7.27 (0.03)	7.26 (0.03)	7.24 (0.02)	7.27 (0.04)	7.28 (0.05)	7.28 (0.04)	N.S.		<0.001		
Mec-Pal	7.20 (0.05)	7.31 (0.06)	7.36 (0.06)	7.31 (0.05)	7.24 (0.02)	7.21 (0.04)	7.24 (0.02)	7.24 (0.02)	7.22 (0.01)	<0.05			N.S.	
Po ₂ (mm Hg)														
Control	101 (28)	106 (21)	67 (5)	64 (3)	64 (5)	77 (8)	88 (9)	85 (8)	89 (6)	N.S.				
Pal	84 (14)	63 (9)	52 (4)	45 (2)	61 (7)	75 (13)	68 (12)	75 (9)	65 (6)	<0.05	0.05			
Mec	147 (23)	115 (24)	64 (7)	54 (9)	52 (5)	63 (9)	88 (13)	77 (10)	73 (10)	<0.001		N.S.		
Mec-Pal	153 (48)	123 (23)	87 (12)	75 (15)	72 (11)	58 (4)	61 (5)	60 (3)	65 (7)	0.01			N.S.	
Pco ₂ (mm Hg)														
Control	45.4 (6)	37.3 (2)	33.6 (2)	37.1 (2)	40.5 (1)	40.9 (3)	37.3 (2)	41.3 (2)	43.1 (1)	N.S.				
Pal	45.6 (9)	42.6 (8)	41.2 (5)	44.3 (5)	42.3 (4)	41.9 (2)	42.5 (3)	44.7 (1)	45.6 (2)	N.S.	N.S.			
Mec	53.9 (7)	42.9 (3)	39.6 (1)	46.6 (2)	51.4 (4)	49.1 (0.5)	46.8 (5)	46.5 (7)	45.3 (6)	N.S.		<0.005		
Mec-Pal	59.8 (9)	43.8 (9)	35.2 (7)	35.7 (3)	43.5 (2)	54.3 (7)	46.8 (4)	46.5 (4)	47.3 (3)	0.01			N.S.	
Sao ₂ (mm Hg)														
Control	98 (2)	99 (0.4)	99 (0.4)	98 (0.2)	97 (0.3)	99 (0.4)	99 (1)	98 (1)	99 (1)	N.S.				
Pal	99 (0.4)	98 (1)	96 (2)	95 (1)	98 (1)	98 (1)	97 (1)	98 (1)	97 (1)	N.S.	N.S.			
Mec	100 (0.2)	99 (1)	97 (1)	92 (3)	91 (5)	93 (3)	95 (5)	92 (6)	90 (7)	<0.01		<0.005		
Mec-Pal	97 (2)	96 (3)	96 (4)	97 (1)	97 (1)	95 (2)	97 (1)	96 (1)	97 (1)	N.S.			N.S.	
Fio ₂														
Control	0.46 (0.07)	0.40 (0.06)	0.33 (0.02)	0.33 (0.03)	0.32 (0.03)	0.33 (0.03)	0.32 (0.02)	0.30 (0.02)	0.29 (0.01)	N.S.				
Pal	0.36 (0.05)	0.34 (0.07)	0.39 (0.08)	0.45 (0.07)	0.53 (0.06)	0.52 (0.06)	0.52 (0.09)	0.56 (0.10)	0.59 (0.14)	N.S.	0.01			
Mec	0.71 (0.08)	0.59 (0.06)	0.45 (0.04)	0.50 (0.05)	0.60 (0.11)	0.64 (0.12)	0.63 (0.13)	0.62 (0.13)	0.60 (0.13)	N.S.		<0.001		
Mec-Pal	0.73 (0.11)	0.68 (0.10)	0.59 (0.14)	0.50 (0.16)	0.51 (0.16)	0.54 (0.16)	0.53 (0.16)	0.54 (0.16)	0.59 (0.14)	N.S.			N.S.	

Values are expressed as mean (SEM).

Pal, palosuran; Mec, meconium; Mec-Pal, meconium-palosuran; N.S., not significant.

Figure 2. Plasma U-II (A) and ET-1 (B) in control (●, *n* = 5) and meconium (■, *n* = 5) groups. The dotted line at 1 h indicates the time of ductal occlusion. Results are expressed as mean ± SEM. **p* < 0.05, ***p* < 0.01, †*p* < 0.001 (between groups).

(Table 2). As a result, over the whole study period, Po₂ was significantly lower in palosuran animals, compared with controls. Furthermore, a tendency toward a reduction in pH and an increase in Fio₂ in palosuran animals resulted in a lower pH and a higher Fio₂ in this group, compared with controls (Table 2).

The increase in U-II that occurred in controls was not altered by palosuran. ET-1 was unchanged in controls but tended to increase during the first postnatal hour in palosuran animals (*p* = 0.07) to levels that were significantly greater than controls beyond this time (*p* < 0.001) (Fig. 4).

Effects of palosuran after meconium aspiration. Between 15 and 360 min after birth, mean P_{PA} was unchanged in the meconium group but fell in the meconium-palosuran group (*p* < 0.001), Q_{LPA} was similar in both groups throughout the study. Thus, although PVR increased in the meconium and meconium-palosuran animals (*p* < 0.005 for both), this increase was significantly attenuated in the latter. By contrast, the increase in OI in the meconium animals was not altered by palosuran (Fig. 5).

The fall in P_{AO} was similar in the meconium and meconium-palosuran animals, but the differences in pulmonary vascular responses between the two groups resulted in an attenuated increase in P_{PA}/P_{AO} in the meconium-palosuran group, compared with the meconium group. After ductal closure, CI was maintained in both meconium and meconium-palosuran groups, and similar falls in SVR occurred in both (*p* < 0.001 for both) (Fig. 5).

ABG variables were similar throughout the study in meconium and meconium-palosuran animals (Table 2).

The postnatal increase in U-II and ET-1 that occurred in the meconium group was not altered by palosuran (Fig. 6).

DISCUSSION

This study has provided two new findings. First, the normal fall in PVR in the initial hours after birth occurred in association with a rise in plasma U-II and was *prevented* by competitive antagonism of the U-II receptor. Second, a postnatal rise in PVR after MAS occurred in association with a greater

Figure 3. Hemodynamics and OI in control (●, $n = 5$) and palosuran (○, $n = 5$) groups. The dotted line at 1 h indicates the time of ductal occlusion. Results are expressed as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, † $p < 0.001$ (between groups).

Figure 4. Plasma U-II (A) and ET-1 (B) in control (●, $n = 5$) and palosuran (○, $n = 5$) groups. The dotted line at 1 h indicates the time of ductal occlusion. Results are expressed as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, † $p < 0.001$ (between groups).

rise in U-II levels and was *attenuated* by antagonism of the U-II receptor.

This study has examined for the first time, the role of U-II in the first hours of life. We have shown that the normal fall in PVR was associated with an increase in U-II. This observation combined with the finding that antagonism of the U-II receptor with palosuran prevented the fall in PVR after birth suggests that U-II-mediated vasodilation contributes to the neonatal reduction in PVR. This hypothesis is consistent with some of the known properties of U-II (19), which are likely to be mediated at least in part through its interaction with specific endothelial receptors (20), resulting in the formation of nitric

oxide, prostanoids, and endothelial-derived hyperpolarizing factor (11,20–22).

Palosuran also produced a striking effect on the systemic circulation with the reduction in P_{AO} suggesting that U-II mediated systemic vasoconstriction may contribute to the maintenance of systemic arterial pressure in the neonatal period.

Our novel transnatal model of MAS closely resembled the human condition, with elevations in PVR and P_{PA} and a reduction in Q_{PA} , alongside an increased OI. By the end of the study, P_{PA} was supra-systemic, reproducing the findings in the clinical setting (23). This differs from previous models of

Figure 5. Hemodynamics and OI in meconium (■, $n = 5$) and meconium-palosan (□, $n = 4$) groups. The dotted line at 1 h indicates the time of ductal occlusion. Results are expressed as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, † $p < 0.001$ (between groups).

Figure 6. Plasma U-II (A) and ET-1 (B) in meconium (■, $n = 5$), and meconium-palosan (□, $n = 4$) groups. The dotted line at 1 h indicates the time of ductal occlusion. Results are expressed as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, † $p < 0.001$ (between groups).

meconium aspiration in more mature animals, which have already negotiated the perinatal transition, with pulmonary pressures peaking at only around a half of systemic (1,5). In one of the few models in the literature to examine, the impact of meconium on the transitional circulation before the animal taking its first breath, PHN failed to develop in neonatal baboons (24). However, in this model, the crucial early postnatal hours were not examined, and Q_{PA} and PVR were not measured. Moreover, only three animals were studied in the meconium group, with right ventricular systolic pressures tending to be higher than in controls.

In our model, we observed that the detrimental effects of meconium aspiration evolved over the first three postnatal hours, before reaching a plateau. This time course was similar to that observed in existing models of MAS (1), and to the clinical setting, when some neonates who may appear well immediately after birth, subsequently decompensate (25). We also demonstrated other features of MAS that are typical in the clinical setting. These included a fall in P_{Ao} (4) and acute parenchymal lung disease leading to ventilation-perfusion mismatch, arterial hypoxemia, acidosis, and an increased OI (1,3,4,26).

The elevation in PVR in MAS was associated with a greater increase in plasma U-II than in controls, and blockade of U-II receptors with palosuran in MAS significantly attenuated the elevation of PVR. These findings suggest that U-II contributes to the increase in PVR in MAS. U-II has been described as the most potent vasoconstrictor yet identified, producing endothelial-independent constriction through its direct interaction with specific receptors on vascular smooth muscle (9). Vasoconstriction is said to predominate in situations where endothelial dysfunction and impaired production of vasodilators coexist, such as in pulmonary hypertension (27). A number of adult models of pulmonary hypertension have supported the contribution of U-II to this condition (10,12). In addition, a recent study of rat pulmonary arteries (22) showed that pulmonary vasoconstrictor responses to U-II were potentiated by ET-1 and by hypoxia, which may partly explain the vasoconstrictor effects of U-II in our model of MAS in the presence of elevated ET-1 levels and arterial hypoxemia.

Our study suggests divergent roles for U-II in the perinatal transition such that under normal circumstances when U-II is relatively low, U-II-mediated vasodilation contributes to the fall in PVR. However, in the disease state, such as MAS, when it is likely that the U-II system is activated (28) and there is a greater increase in U-II, vasoconstriction may predominate. This hypothesis is partly supported by studies in rat aortic strips, where low concentrations of U-II caused transient relaxation, while higher concentrations caused sustained contraction (29).

A compelling analogy may be drawn between these observations and the properties of ET-1. Like U-II, ET-1 produces both endothelium-dependent vasodilation and endothelium-independent vasoconstriction (11,30) and plasma ET-1 levels are increased in the perinatal transitional circulation in some species (31). Although controversial, some investigators have proposed that ET-1-mediated vasodilation contributes to the changes in PVR during the early neonatal period (31). By contrast, in MAS, a heightened increase in ET-1 contributes to the genesis of pulmonary hypertension (2,7,8).

Limitations. Our observation that palosuran abolished the fall in PVR led us to propose a primary, receptor-mediated role for U-II in the neonatal pulmonary vasodilator response. However, it must be acknowledged that administration of palosuran was associated with effects on blood gases, which could have contributed to the blunted pulmonary vasodilator response observed in the palosuran group. PO_2 was slightly lower during the study period in this group than controls. We consider that this is unlikely to contribute to the blunted pulmonary vasodilator response, for a number of reasons: first, the differences were modest, Sao_2 was well maintained; and second, in related models of the perinatal transitional circulation, neonatal pulmonary vasodilator responses occurred in the presence of much more extreme levels of hypoxemia (30,32). Consideration also needs to be given to whether the differences in pH between control and palosuran animals contributed to the blunted response. Again, we do not consider that these differences have made a significant contribution, as changes in PVR occurred while pH in the palosuran group remained unchanged over the entire study period, and the

major rise in PVR occurred early in the study, when pH was stable in both groups.

As the primary purpose of this article was to examine the pulmonary vascular effects of U-II, ventricular contractility was not directly measured. However, U-II is known to have both positive and negative inotropic effects (9,11), therefore, it is possible that a negative inotropic or direct cardiotoxic effect of U-II antagonism in the normal perinatal circulation may have contributed to the systemic hemodynamic changes observed in palosuran treated controls.

Finally, it is important to note that our study was designed to examine the physiologic relevance of the endogenous U-II system in the perinatal circulation in the absence and presence of meconium aspiration. As a result, our experimental paradigm measured endogenous U-II, and the effects of a U-II receptor blocker, rather than the actions of exogenous infusions of U-II *per se*.

In conclusion, U-II, the recently characterized vasoactive peptide, appears to play a divergent role in the transitional circulation, contributing to the fall in PVR under normal circumstances, whereas producing pulmonary vasoconstriction during PHN secondary to MAS. Blockade of the U-II receptor with palosuran improves pulmonary hemodynamics in this model of MAS. Further studies are required to confirm our findings.

Acknowledgments. We thank Andrew Hattam and Magdy Sourial for assistance with experimental studies, Jonathan Mynard for developing our software, A/Prof Tim Yandle, Steve Fisher, and the Cardioendocrine Research Group, Christchurch, New Zealand, for performing the assays, and Dr Martine Clozel and Dr Marc Iglarz from Actelion Pharmaceuticals for supplying palosuran.

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