Paper

Muscarinic effects and foetal cardiovascular and hormonal responses in utero

Jianli Zheng,[1] *Weili Yang,*[1] *Li Cao, Shigang Li, Yuying Zhang, Zhen Wan, Caiping Mao*

Key words: aldosterone, angiotensin-converting enzyme, autonomic regulation, foetus

[1] Jianli Zheng and Weili Yang contributed equally to this work.

Perinatal Biology Centre, Soochow University School of Medicine, Suzhou 215123, People's Republic of China.

Correspondence to:
Caiping Mao, PhD
Perinatal Biology Centre, Soochow University School of Medicine, Suzhou 215123, People's Republic of China.
Tel: +86 512 6588 0125
Fax: +86 512 6588 0103
Email: maocaiping@suda.edu.cn

Journal of the Renin-Angiotensin-Aldosterone System
(Including other Peptidergic systems)

September 2009
Volume 10
Number 3

Abstract

Introduction. Cholinergic mechanisms play an important role in the control of hormonal and vascular regulation. However, *in utero* development of cholinergic regulation in the foetal hormonal systems is not clearly understood. This study investigated foetal hormonal and cardiovascular responses following application of the muscarinic antagonist atropine.
Materials and methods. Chronically prepared near-term ovine foetuses (control and experimental: n=5, each group) were used. After 4–5 days' surgical recovery, conscious ewes and their foetuses were tested *in vivo*.
Results. In response to intravenous atropine, foetal systolic, diastolic, and mean arterial pressure, as well as heart rate, increased immediately. Inhibition of muscarinic systems in the circulation caused a reduction of plasma angiotensin II levels, while angiotensin I in the circulation remained unchanged in the foetus. In addition, foetal plasma aldosterone levels were significantly increased following blockade of the cholinergic receptor, while other hormones, including arginine vasopressin, adrenocorticotrophic hormone, and atrial natriuretic peptide, were not changed in foetal blood under the same condition.
Conclusions. The results suggest that foetal automatic systems, not those hormonal factors tested, play a major role in cholinergic mechanisms mediating cardiovascular control. Furthermore, the data provide new information on how muscarinic inhibition affects renin-angiotensin system and adrenal cortex functions.
Key words: aldosterone, angiotensin-converting enzyme, autonomic regulation, foetus

Introduction

During pregnancy, many environmental factors may influence foetuses *in utero* via cholinergic changes. For example, smoking in pregnancy causes exposure of pregnant mothers to nicotine that can penetrate the placental barrier,[1-3] and enter the foetus. Numerous studies have shown that cholinergic mechanisms are important in the physiological control of the vascular and hormonal systems.[4-6] Peripheral application of atropine, a non-selective muscarinic receptor antagonist, produces an increase of blood pressure and heart rate in adults.[7] Little is known about peripheral cholinergic mechanisms in the regulation of vascular and endocrine functions in the foetus. Addressing this question is important not only for pre-natal life, but also for post-natal health.

In general, parasympathetic postganglionic neurons release acetylcholine and sympathetic postganglionic neurons release noradrenaline. The transmission between pre- and postganglionic neurons in parasympathetic nerve is cholinergic.[8,9] The autonomic system plays an important role in cardiovascular regulation in adults.[10,11] Whether and to what extent cholinergic mechanisms are involved in regulation of the foetal automatic system in the control of cardiovascular functions *in utero* is not clear. Considering both automatic and hormonal systems acting as two major mechanisms in the control of blood pressure and heart rate,[12,13] it is necessary to examine hormonal influences in the study of cholinergic regulation of the foetal cardiovascular responses. Among many hormones or compounds, angiotensin (Ang), arginine vasopressin (AVP), adrenocorticotrophic hormone (ACTH), atrial natriuretic peptide (ANP), aldosterone, and prostaglandin E (PGE) have been considered critical in the control of either cardiovascular activity or vascular volume.[14,15] Therefore, these hormones and compounds were determined in the present foetal study *in vivo* and *in utero*. The present study used the muscarinic (M) receptor antagonist in determination of the role of cholinergic mechanisms in hormonal and vascular regulation in the ovine foetus.

10.1177/1470320309104866

Materials and methods

Time-dated pregnant ewes with foetuses (131±3 days of gestation on the study day; term for sheep is ~ 147 days) were used. Animals were housed in individual study cages and in a light-controlled room (12:12-h light-dark cycle) with food and water provided *ad libitum*. All surgical and experimental procedures had been approved by the Institute Animal Care Service.

Surgical preparation

Anaesthesia was induced with ketamine hydrochloride (20 mg/kg, i.m.; Hengrui Medicine, JiangSu, People's Republic of China), and general anaesthesia was maintained with 3% isoflurane and 1 L/min oxygen. The uterus was exposed by midline abdominal incision, and a small hysterotomy was performed to provide access to a foetal hindlimb as reported previously.[16,17] Polyethylene catheters were placed in the maternal and foetal femoral vein and artery and advanced to the inferior vena cava and abdominal aorta, respectively. The foetus was then returned into the uterus, and the hysterotomy was closed in two layers. All catheters were externalised to the maternal flank and placed in a cloth pouch. Animals recovered for 4 days after surgery. Immediately pre-operatively and twice daily during the initial 3 days of recovery, gentamicin (8 mg; North China Pharmaceutical, People's Republic of China) and oxacillin (30 mg) were administered intravenously to the foetus, and gentamicin (70 mg) and oxacillin (1 g) were injected intravenously into the ewe.

Cardiovascular experiments

On the testing day, sheep were allowed a period of 60–100 minutes to acclimatise to the testing rooms. When animal heart rates and arterial pressures appeared to be stable, a 60-minute baseline was followed by 20 minutes of intravenous infusion and an additional 90-minute period. Foetal and maternal systolic, diastolic, and mean arterial pressure (MAP) were monitored using a PowerLab physiological recorder with Chart-5 software (AD Instruments, Australia). Animals were divided into control (n=5) and experimental (n=5) groups with computer-randomised selection. Beginning at time 0, atropine (0.033 mg/kg in 10 ml 0.9% NaCl; Sigma-Aldrich, St. Louis, MO, USA) was infused intravenously to the experimental foetus over 20 minutes. The dose of intravenous atropine was selected according to previous reports.[18] For the control animals, the same volume of isotonic saline (vehicle) was infused intravenously. Maternal and foetal blood pressure and heart rate were monitored during the testing period. Blood pressure and heart rate were determined by computer analysis of waveforms by utilising a customised pattern recognition algorithm.

Blood value experiments

Maternal and foetal blood samples were collected at –30 and 0 minutes of the baseline period and at 5, 30, and 60 minutes after intravenous infusion of atropine or vehicle. All foetal blood samples (4 ml/sample) were replaced with equivalent volumes of heparinised maternal blood withdrawn before the study, and all maternal blood samples were replaced with equivalent volumes of isotonic saline. Blood samples were withdrawn from the foetal and maternal arterial catheters for measurements of blood PO_2, PCO_2, haemoglobin, haematocrit, electrolytes, lactic acid, and pH on a Nova analyser system (model pHOx Plus L; Nova Biochemical, Waltham, MA, USA) adjusted to sheep internal temperature (39°C). Plasma osmolality was measured by using freezing point depression on an advanced digimatic osmometer (model 3MO; Advanced Instruments, Needham Heights, MA, USA).

Endocrine experiments

Foetal blood samples were collected into iced tubes containing lithium heparin during the baseline and study periods. Blood samples for hormone assays were centrifuged immediately. The plasma was then stored at –20°C before measurement of hormone using radioimmunoassay. Plasma samples for radioimmunoassay and data analysis were handled in a blind manner.

Data analysis

Foetal and maternal blood pressure and heart rate were determined by computer analysis of waveforms utilising a customised pattern recognition algorithm. All signals in cardiovascular studies were digitised at 500 Hz and recorded on a computer with Chart-5 acquisition software. Heart rate, systolic and diastolic pressure, and MAP were calculated from the pressure waveforms by means of Power-Lab software. Repeated measures ANOVA followed by post-hoc tests (Tukey's test) was used to determine differences over time and effects of the treatments. All data are expressed as mean±SEM.

Results

Cardiovascular responses

There was no significant difference between the control and experimental groups, and between before and after administration of atropine, in maternal systolic pressure, diastolic pressure, MAP, and heart rate (all $p>0.05$; table 1). However, intravenous infusion of atropine (0.033 mg/kg in 10 ml saline) into the foetus significantly increased foetal systolic, diastolic, and mean arterial pressure

Journal of the Renin-Angiotensin-Aldosterone System
(Including other Peptidergic systems)

September 2009
Volume 10
Number 3

SAGE Publications 2009 Los Angeles, London, New Delhi and Singapore

Table 1
Maternal cardiovascular responses before and after intravenous infusion of atropine (0.033 mg/kg) into the foetus.

		Time before and after atropine injection				
Value	**Group**	**Baseline**	**0 min**	**5 min**	**30 min**	**60 min**
SP	I	115.73±3.59	115.79±4.23	116.49±4.37	115.82±4.89	119.79±4.74
(mmHg)	II	115.15±2.78	116.23±1.56	116.25±2.65	115.68±4.73	116.26±4.72
DP	I	83.75±3.14	84.21±2.47	82.09±4.17	84.51±4.37	83.96±3.45
(mmHg)	II	82.76±3.36	83.75±2.81	83.50±3.68	83.41±4.03	82.43±3.36
MAP	I	95.82±2.72	96.56±2.77	95.81±3.74	98.17±3.96	97.52±3.08
(mmHg)	II	95.18±2.37	96.47±3.82	95.26±3.15	97.02±3.36	96.25±2.63
HR	I	121.56±7.13	125.71±8.37	124.69±7.74	123.43±7.63	125.58±7.36
(bpm)	II	120.59±6.78	125.08±7.63	126.79±6.46	124.53±7.59	127.13±7.06

Key: Values are mean±SEM. DP = diastolic pressure; HR = heart rate; I = intravenous infusion of atropine (0.033 g/kg); II = intravenous infusion of vehicle (0.9% NaCl); MAP = mean arterial pressure; SP = systolic pressure.

as compared to the control foetuses (figure 1). Foetal MAP was increased from the baseline level, 48.8±0.4 mmHg to 57.6±0.3 mmHg, within 5 minutes after intravenous administration of atropine ($p<0.01$). In the control animals, intravenous administration of vehicle had no effect on foetal systolic, diastolic, and mean arterial pressure in the near-term foetus (all $p>0.05$). Immediately following intravenous infusion of atropine, foetal heart rate was significantly increased (figure 2).

Blood values

There was no significant difference in foetal arterial blood pH, PO_2, PCO_2, haemoglobin, and haematocrit before or after intravenous infusion of atropine (table 2). For both the control and experimental animals, intravenous infusion of atropine or vehicle had no effect on plasma osmolality in the foetal animals. Foetal K^+ and Na^+ concentrations were not changed. All arterial values were within physiological ranges and did not vary significantly between the control and experimental groups (all p values not significant; table 2). In the maternal sheep, intravenous infusion of atropine or vehicle into the foetus had no effect on plasma osmolality, Na^+ and K^+ concentrations, blood pH, PO_2, and PCO_2 (all p values not significant; table 3).

Plasma hormone assay

Administration of intravenous atropine did not affect foetal plasma Ang I levels; however, plasma Ang II concentrations were significantly lowered (figure 3). In addition, intravenous infusion of atropine significantly increased plasma aldosterone levels in the foetus, while foetal AVP concentrations remained unchanged in plasma (figure 4). Administration of atropine also had no effect on plasma ACTH, ANP, and PGE2 levels in the foetus at near term (table 4).

Discussion

Although cholinergic systems are important in the control of cardiovascular and hormonal regulation,[19-22] there have been limited data on the functional development of cholinergic mechanisms in regulation of foetal vascular and endocrine systems. In the present study, we found that cholinergic inhibition by intravenous atropine affected foetal blood pressure in association with a decrease of Ang II and increase of aldosterone concentrations in the foetal circulation.

Atropine is known as a potent and competitive muscarinic receptor antagonist. Previous studies in mature animals have shown that atropine-induced cardiovascular responses were of short duration.[23-25] In the present study, the onset of the intravenous atropine-induced blood pressure responses in the foetus was rapid. The foetal systolic pressure, diastolic pressure, and MAP were significantly increased after intravenous administration of atropine. The increased foetal blood pressure returned to the baseline within 30 minutes. It appeared that atropine acted directly on M receptors in cardiovascular systems, blocking acetylcholine from parasympathetic postganglionic neurons in binding the M receptors on vascular smooth muscle, and caused an increase of blood pressure. The foetal heart rate also increased immediately, and reached maximum in 5 minutes after administration of the drug. The involvement of the autonomic nervous system in the control of cardiovascular responses following administration of cholinergic antagonists has been thoroughly investigated in adult animals.[23] Atropine could block the muscarinic receptors of parasympathetic postganglionic pathways, resulting in a comparable increase of sympathetic activity. The cardiovascular data in the present study demonstrate that cholinergic mechanisms in the control of the foetal autonomic nervous system are developed and functional, at least at near term, *in utero*.

SAGE Publications 2009 Los Angeles, London, New Delhi and Singapore

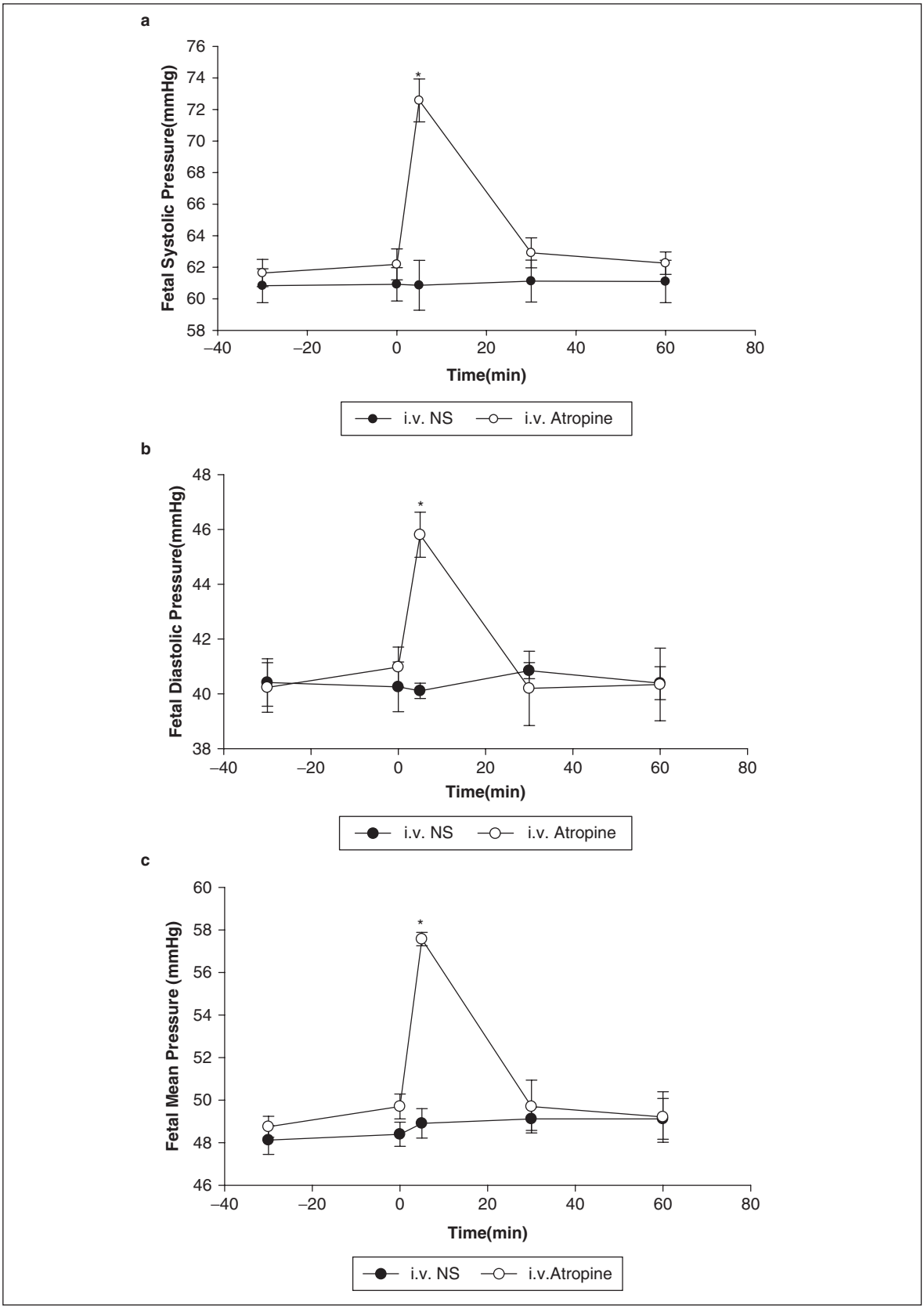

Figure 1
Intravenous atropine increased systolic (a), diastolic (b), and mean arterial pressure (c) in the ovine foetus at near term. *p<0.05 *vs.* baseline. i.v. Atropine = intravenous atropine; i.v. NS = intravenous 0.9% NaCl solution; 0 min = time before intravenous injection (baseline).

An earlier autoradiographic study has shown that an active cholinergic system is present in the foetus.[26,27] The present study demonstrates that cholinergic antagonist atropine in the foetal circulation could cause a short period of pressor responses by inhibition of M receptors, indicating that foetal muscarinic systems play a critical role in maintenance of normal basal blood pressure *in utero* at near term; and

Journal of the Renin-Angiotensin-Aldosterone System
(Including other Peptidergic systems)

September 2009
Volume 10
Number 3

SAGE Publications 2009 Los Angeles, London, New Delhi and Singapore

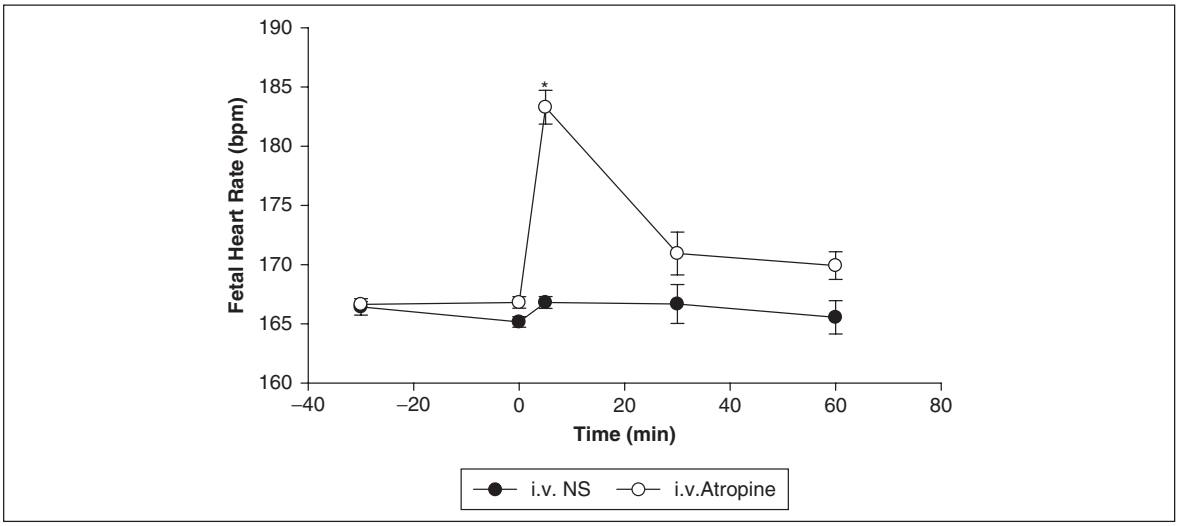

Figure 2
Intravenous atropine increased ovine foetal heart rate at near term. *p<0.05 vs. baseline. i.v. Atropine = intravenous atropine; i.v. NS = intravenous 0.9% NaCl solution; 0 min = time before intravenous injection (baseline).

that cholinergic inhibitory mechanisms in the control of foetal blood pressure at physiological ranges are established before birth.

Apart from autonomic pathways in the control of cardiovascular changes, humoral mechanisms also play an important role in cardiovascular regulation via their actions on vascular tension or vessel volume.[28-30] Among these chemicals, Ang I, Ang II, AVP, ACTH, ANP, aldosterone, and PGE are crucial factors that can either directly affect cardiovascular activity or influence vascular

Table 2
Foetal arterial values before and after intravenous infusion of atropine or vehicle into the foetus.

	Time before and after atropine injection		
Value	**Baseline**	**5 min**	**60 min**
ACTH (pg/ml)	32.2±7.6	34.6±4.4	35.0±10.2
PGE2 (pg/ml)	43.6±7.5	53.0±2.8	53.8±6.6
ANP (pg/ml)	345.7±15.1	362.8±15.2	319.7±25.0

Key: Values are mean±SEM. ACTH = adrenocorticotrophic hormone; ANP = atrial natriuretic peptide; PGE2 = prostaglandin E.

Table 3
Maternal arterial values before and after intravenous infusion of atropine or vehicle into the foetus.

		Time before and after atropine injection		
Value	**Group**	**Baseline**	**5 min**	**60 min**
pH	I	7.47±0.01	7.47±0.01	7.48±0.01
	II	7.46±0.01	7.47±0.01	7.48±0.01
PCO_2 (mmHg)	I	40.80±1.38	39.06±1.48	39.14±0.60
	II	38.09±0.71	39.63±1.65	37.20±0.74
PO_2 (mmHg)	I	20.02±2.85	20.94±1.05	19.88±1.19
	II	20.15±2.15	19.59±2.20	21.30±2.78
Hb (g/dl)	I	8.60±0.10	8.30±0.24	8.18±0.26
	II	8.63±0.18	8.50±0.17	8.63±0.09
Hct (%)	I	27.00±0.45	27.60±0.60	26.8±0.86
	II	27.33±0.33	26.67±0.88	27.30±0.33
Na^+ (meq/L)	I	136.76±0.78	138.10±1.06	138.10±1.06
	II	139.20±1.28	139.00±1.30	139.17±1.33
K^+ (meq/L)	I	4.30±0.19	4.27±0.18	4.32±0.18
	II	4.08±0.80	3.86±0.59	3.94±0.75
Osmolality (mosmol/kg)	I	294.00±4.75	318.80±12.33	299.20±4.03
	II	290.33±5.61	294.33±10.04	292.00±5.69
Glu (mmol/L)	I	1.83±0.38	1.90±0.28	1.90±0.32
	II	1.82±0.19	1.76±0.18	1.78±0.17
Lac (mmol/L)	I	1.50±0.06	1.40±0.01	1.40±0.07
	II	1.60±0.10	1.65±0.05	1.55±0.05

Key: Values are mean±SEM. Glu = glucose; Hb = haemoglobin; Hct = haematocrit; I = intravenous infusion of atropine (0.033 g/kg); II = intravenous infusion of vehicle (0.9% NaCl); Lac = lactic acid.

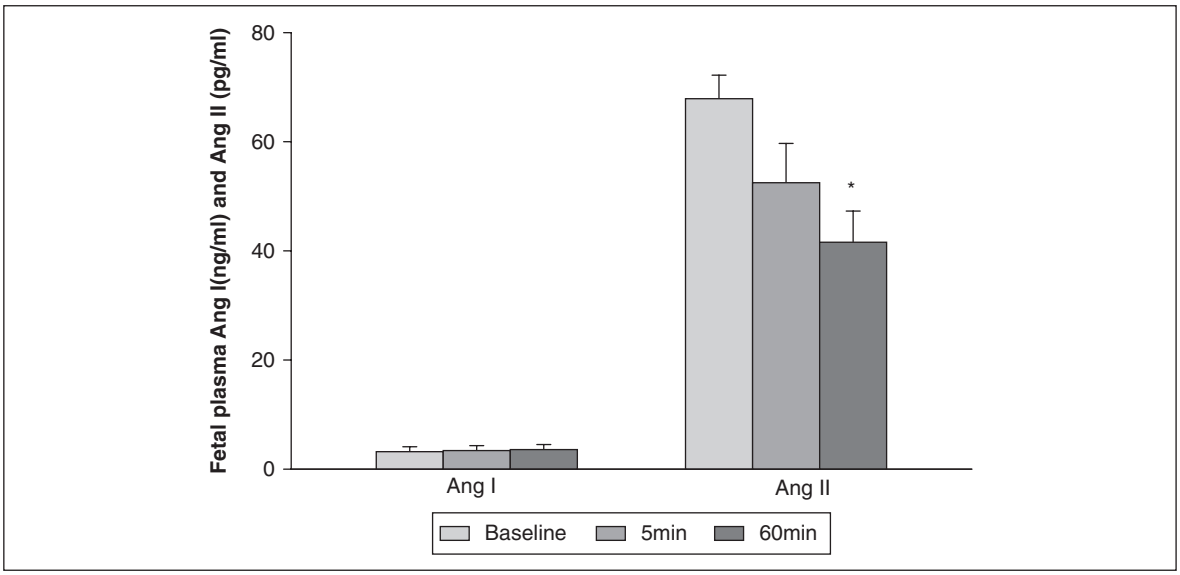

Figure 3
The effect of intravenous atropine on plasma Ang I and Ang II levels in the ovine foetus at near term. *p<0.05 vs. baseline. Ang = angiotensin; 5 min and 60 min = time after intravenous injection.

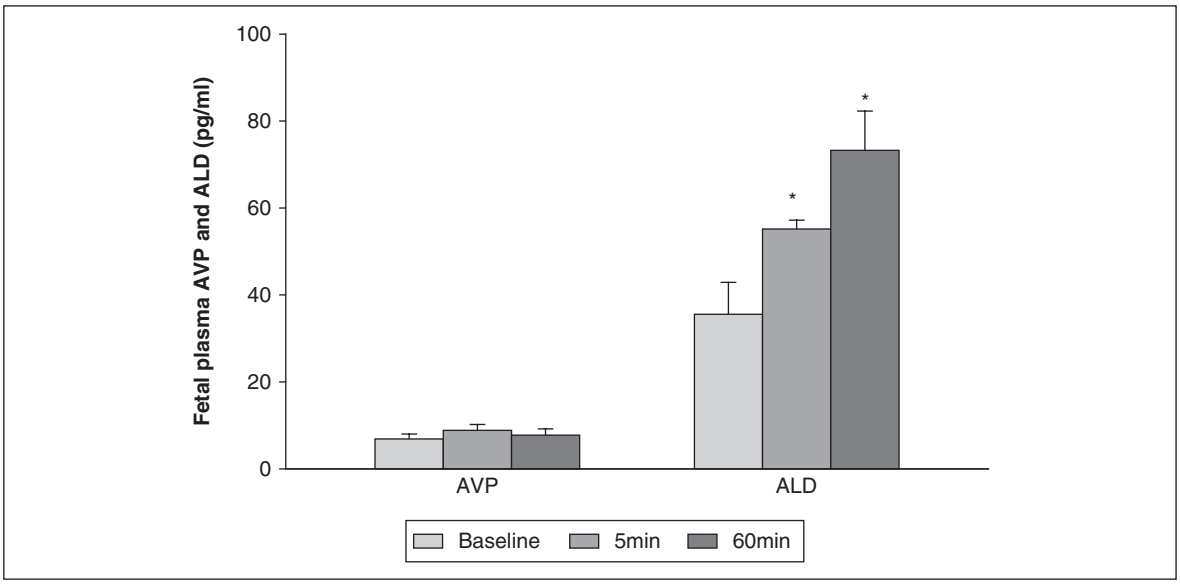

Figure 4
The effect of intravenous atropine on plasma AVP and ALD levels in the ovine foetus at near term. *p<0.05 vs. baseline. ALD = aldosterone; AVP = arginine vasopressin; 5 min and 60 min = time after intravenous injection.

volume.[14,15] In the present study, we measured these seven hormones or compounds in the foetal circulation after the treatment with atropine. The data showed that foetal plasma Ang II levels were decreased, and aldosterone concentrations were increased. Although AVP, ACTH, ANP, and PGE can also affect cardiovascular responses and body fluid balance,[14,15] we did not find any change in these humoral levels in the foetus after administration of atropine.

The renin-angiotensin system (RAS) is important in the regulation of blood pressure and in the development of some forms of clinical and experimental hypertension.[31,32] Previous studies have shown that an increase of blood pressure was related to an increase of plasma Ang II.[33,34] In the present study, we observed a decrease of foetal plasma Ang II following administration of atropine, in suggestion that formation of Ang II in the foetal circulation was down regulated after blockade of muscarinic systems. Although many factors may affect production of Ang II in the body, plasma Ang I levels, which mirror renin activity or production of angiotensinogen, were not changed in the foetus under the same condition. This indicates that the inhibition for Ang II production was a subsequent event after

Table 4
Foetal plasma hormone levels before and after intravenous infusion of atropine into the foetus

Value	Group	Time before and after atropine injection		
		Baseline	5 min	60 min
pH	I	7.46±0.01	7.45±0.02	7.47±0.01
	II	7.47±0.01	7.47±0.01	7.47±0.01
PCO_2 (mmHg)	I	24.35±0.98	24.97±1.67	25.93±1.27
	II	24.01±1.51	25.61±0.23	23.91±1.78
PO_2 (mmHg)	I	115.65±2.34	117.35±2.23	115.90±2.71
	II	115.38±1.57	113.02±2.70	117.76±6.57
SO_2 (%)	I	98.51±0.23	98.34±0.36	97.42±0.75
	II	98.43±0.22	99.05±0.45	98.73±0.15
Hb (g/dl)	I	10.18±0.32	10.20±0.40	9.42±0.75
	II	9.97±0.19	10.20±0.20	9.87±0.32
Hct (%)	I	29.60±0.81	30.60±1.21	28.40±2.23
	II	28.24±0.59	29.00±1.12	28.67±1.20
Na^+ (meq/L)	I	143.66±0.45	144.28±0.60	144.46±0.70
	II	144.20±1.07	144.65±1.15	145.03±1.75
K^+ (meq/L)	I	3.87±0.03	3.80±0.10	3.57±0.28
	II	3.89±0.07	4.05±0.03	3.90±0.13
Osmolality	I	301.20±2.75	302.00±3.79	308.00±2.77
(mosmol/kg)	II	301.67±3.18	302.33±1.85	303.00±2.08
Glu (mmol/L)	I	4.49±0.08	3.39±0.09	3.06±0.26
	II	4.38±0.19	4.63±0.43	4.45±0.49

Key: Values are mean±SEM. Glu = glucose; Hb = haemoglobin; Hct = haematocrit; I = intravenous infusion of atropine (0.033 g/kg); II = intravenous infusion of vehicle (0.9% NaCl).

formation of Ang I. Angiotensin-converting enzyme (ACE) is a key element for turning Ang I into Ang II in the RAS in general.[35] Previous studies have demonstrated that ACE release could be regulated by cholinergic mechanisms.[36] The present study is the first in foetal studies to show that muscarinic inhibition can down regulate the circulating RAS by possible mechanisms in inhibiting ACE. Although a down-regulated RAS does not support the hypothesis that the increased foetal blood pressure in the present study was due to the change of plasma Ang II, the finding that cholinergic inhibition of the foetal peripheral RAS at ACE level *in utero* is interesting. Furthermore, the data from the foetal RAS analysis add supportive information that autonomic mechanisms may play a major role in the positive cardiovascular response to blockade of cholinergic pathways in the foetus.

The mineralocorticoid hormone aldosterone is considered to affect cardiovascular functions via vascular volume regulation, by its action to retain sodium at epithelial tissues.[37,38] In the present study, foetal plasma aldosterone was significantly increased following intravenous administration of atropine. Did an increase of foetal plasma aldosterone contribute to the increase of blood pressure in the present study? We monitored blood haematocrit and haemoglobin concentrations throughout the testing periods, and did not find any significant change in the circulation, indicating that blood volume remained unchanged. Together, the foetal cardiovascular response induced by the cholinergic blocker was unlikely to be linked to hormonal mechanisms investigated in the present study.

What could be the mechanism for intravenous atropine-increased foetal aldosterone in the circulation? At the time of testing, we monitored blood electrolytes and osmolality levels in both maternal and foetal sheep. There was no change of sodium and osmolality in either the mother or the foetus following administration of atropine. This does not support the possibility that atropine-increased foetal aldosterone might be due to osmotic regulatory mechanisms. The renin-angiotensin-aldosterone pathway[39] is also excluded because the change of foetal aldosterone was opposite to that of Ang II. Previous studies have demonstrated that following intravenous administration of a relatively selective M receptor antagonist pirenzepine, or a non-selective muscarinic antagonist atropine, aldosterone in the circulation increased significantly in adults with both regimens.[5] In addition, there was evidence of a direct cholinergic influence on adrenal zona glomerulosa functions.[6] Thus, after excluding other major possible influences, we suggest that cholinergic regulation directly in the adrenal cortex may be the reason for cholinergic antagonist-increased foetal aldosterone release.

Conclusion

In conclusion, the present study demonstrates novel information that a normal cholinergic activation and muscarinic basal tone is important in maintenance of foetal blood pressure at basal levels before birth. Inhibition or suppression of M receptors in the circulation will lead to an acute increase of cardiovascular responses *in utero*, mainly via autonomic pathways. Interestingly, the present study also provides new findings that muscarinic inhibition in the circulation can suppress the peripheral RAS and stimulate adrenal zona glomerulosa functions in the foetus *in utero*. Considering many environmental factors such as smoking during pregnancy may affect cholinergic activation *in utero*, the findings in the present study may be important for both pre-natal development and post-natal health.

Acknowledgements

Jianli Zheng and Weili Yang contributed equally to this work. This work was supported in part by the National Natural Science Foundation (No.30570915, No.30871400), Jiangsu Natural Science Key Grant (BK2006703), Jiangsu High Education NSF (08KJB320013), Suzhou Key Lab Grant (SZS0602), Suda Programme Project Grant (No.90134602), Suzhou International Cooperation Grant (N2134703), and Suda Medical Key Grant (EE134704).

References

1. Slotkin TA. Fetal nicotine or cocaine exposure: which one is worse? *Pharmacol Exp Ther* 1998;**285**:931-45.
2. Yuan H, Platt RW, Morin L, Joseph KS, Kramer MS. Fetal deaths in the United States, 1997 vs 1991. *Am J Obstet Gynecol* 2005;**193**:489-95.
3. Zdravkovic T, Genbacev O, McMaster MT, Fisher SJ. The adverse effects of maternal smoking on the human placenta: a review. *Placenta* 2005;**26**(suppl A):S81-6.
4. Lee SB, Kim SY, Sung KW. Cardiovascular regulation by cholinergic mechanisms in rostral ventrolateral medulla of spontaneously hypertensive rats. *Eur J Pharmacol* 1991;**205**:117-23.
5. Sommers DK, Meyer EC, van Wyk M. The effect of neostigmine on metoclopramide-induced aldosterone secretion after the administration of muscarinic antagonists in man. *Eur J Clin Pharmacol* 1990;**38**:401-03.
6. Jánossy A, Orsó E, Szalay KS *et al*. Cholinergic regulation of the rat adrenal zona glomerulosa. *J Endocrinol* 1998;**157**:305-15.
7. Poller U, Nedelka G, Radke J, Pönicke K, Brodde OE. Age-dependent changes in cardiac muscarinic receptor function in healthy volunteers. *J Am Coll Cardiol* 1997;**29**:187-93.
8. Lindh B, Hokfelt T. Structural and functional aspects of acetylcholine peptide coexistence in the autonomic nervous system. *Prog Brain Res* 1990;**84**:175-91.
9. De Groat WC, Booth AM. Inhibition and facilitation in parasympathetic ganglia of the urinary bladder. *Fed Proc* 1980;**39**:2990-6.
10. Iwasaki K, Zhang R, Zuckerman JH, Levine BD. Dose-response relationship of the cardiovascular adaptation to endurance training in healthy adults: how much training for what benefit? *J Appl Physiol* 2003;**95**:1575-83.
11. Galanter CA, Wasserman G, Sloan RP, Pine DS. Changes in autonomic regulation with age: implications for psychopharmacologic treatments in children and adolescents. *J Child Adolesc Psychopharmacol* 1999;**9**:257-65.
12. Terzolo M, Matrella C, Boccuzzi A *et al*. Twenty-four hour profile of blood pressure in patients with acromegaly. Correlation with demographic, clinical and hormonal features. *J Endocrinol Invest* 1999;**22**:48-54.
13. Oświecimska J, Ziora K, Adamczyk P *et al*. Effects of neuroendocrine changes on results of ambulatory blood pressure monitoring (ABPM) in adolescent girls with anorexia nervosa. *Neuro Endocrinol Lett* 2007;**28**:410-16.
14. Rademaker MT, Charles CJ, Espiner EA *et al*. Beneficial hemodynamic, endocrine, and renal effects of urocortin in experimental heart failure: comparison with normal sheep. *J Am Coll Cardiol* 2002;**40**:1495-505.
15. Shi L, Yao J, Koos BJ, Xu Z. Induced fetal depressor or pressor responses associated with c-*fos* by intravenous or intracerebroventricular losartan. *Dev Brain Res* 2004;**153**:53-60.
16. Xu Z, Glenda C, Day L, Yao J, Ross MG. Central angiotensin induction of fetal brain c-fos expression and swallowing activity. *Am J Physiol Regul Integr Comp Physiol* 2001;**280**:R1837-43.
17. Xu Z, Nijland MJ, Ross MG. Plasma osmolality dipsogenic thresholds and c-fos expression in the near-term ovine fetus. *Pediatr Res* 2001;**49**:678-85.
18. Naguib M, Gomaa M. Atropine-neostigmine mixture: a dose-response study. *Can J Anaesth* 1989;**6**:412-17.
19. Brezenoff HE, Giuliano R. Cardiovascular control by cholinergic mechanisms in the central nervous system. *Annu Rev Pharmacol Toxicol* 1982;**22**:341-81.
20. Barbosa SP, de Gobbi JI, Zilioli L *et al*. Role of cholinergic and adrenergic pathways of the medial septal area in the water intake and pressor response to central angiotensin II and carbachol in rats. *Brain Res Bull* 1995;**37**:463-6.
21. Harding JW, Jensen LL, Quirk WS, Dewey AL, Wright JW. Brain angiotensin: critical role in the ongoing regulation of body fluid homeostasis and cardiovascular function. *Peptides* 1989;**10**:261-4.
22. Breen S, Rees S, Walker D. Identification of brainstem neurons responding to hypoxia in fetal and newborn sheep. *Brain Res* 1997;**748**:107-21.
23. Ali-Melkkila T, Kanto J, Iisalo E. Pharmacokinetics and related pharmacodynamics of anticholinergic drugs. *Acta Anaesthesiol Scand* 1993;**37**:633-42.
24. Lazartigues E, Brefel-Courbon C, Tran MA, Montastruc JL, Rascol O. Spontaneously hypertensive rats cholinergic hyperresponsiveness: central and peripheral pharmacological mechanisms. *Br J Pharmacol* 1999;**127**:1657-65.
25. Saito R, Kamiya HO, Ono N. Role of the central muscarinic receptor of prostaglandin I2 in cardiovascular function in rat. *Brain Res* 1985;**330**:167-9.
26. Kinney HC, O'Donnell TJ, Kriger P, White WF. Early developmental changes in [3H]nicotine binding in the human brainstem. *Neuroscience* 1993;**55**:1127-38.
27. Kinney HC, Panigrahy A, Rava LA, White WF. Three-dimensional distribution of [3H]quinuclidinyl benzilate binding to muscarinic cholinergic receptors in the developing human brainstem. *J Comp Neurol* 1995;**362**:350-67.
28. Volpe M, Musumeci B, De Paolis P, Savoia C, Morganti A. Angiotensin II AT2 receptor subtype: an uprising frontier in cardiovascular disease? *J Hypertens* 2003;**21**:1429-43.
29. Pratt RE. Angiotensin II and the control of cardiovascular structure. *J Am Soc Nephrol* 1999;**10**(suppl 11):120-8.
30. Riegger GA, Albert M, Kochsiek K. Cardiovascular effects of AVP and ANG in experimental pulmonic stenosis in rats. *Am J Physiol* 1988;**254**:H438-42.

31. Reid IA. Interactions between ANG II, sympathetic nervous system, and baroreceptor reflexes in regulation of blood pressure. *Am J Physiol* 1992;**262**:E763-78.
32. Guillery EN, Robillard JE. The renin-angiotensin system and blood pressure regulation during infancy and childhood. *Pediatr Clin North Am* 1993; **40**:61-79.
33. Hall JE. The renin-angiotensin system: renal actions and blood pressure regulation. *Compr Ther* 1991;**17**:8-17.
34. Mitsui T, Nomura S, Itakura A, Mizutani S. Role of aminopeptidases in the blood pressure regulation. *Biol Pharm Bull* 2004;**27**:768-71.
35. Scott BB, McGeehan GM, Harrison RK. Development of inhibitors of the aspartyl protease renin for the treatment of hypertension. *Curr Protein Pept Sci* 2006; **7**:241-54.
36. Ondetti MA, Rubin B, Cushman DW. Design of specific inhibitors of angiotensin-converting enzyme: new class of orally active antihypertensive agents. *Science* 1977; **196**:441-4.
37. Young MJ, Funder JW. Mineralocorticoid receptors and pathophysiological roles for aldosterone in the cardiovascular system. *J Hypertens* 2002;**20**:1465-8.
38. Fuller PJ. Aldosterone and its mechanism of action: more questions than answers. *Aust N Z J Med* 1995; **25**:800-07.
39. Johnston CI, Hodsman PG, Kohzuki M, Casley DJ, Fabris B, Phillips PA. Interaction between atrial natriuretic peptide and the renin angiotensin aldosterone system. Endogenous antagonists. *Am J Med* 1989;**87**:24S-28S.