TRANSLATIONAL PHYSIOLOGY

Sodium tanshinone IIA sulfonate increased intestinal hemodynamics without systemic circulatory changes in healthy newborn piglets

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Departments of [1]Pediatrics, [3]Obstetrics and Gynecology, and [4]Surgery, University of Alberta, Edmonton, Alberta, Canada; [2]Department of Pediatrics, Children's Hospital of Fudan University, Shanghai; and [5]Department of Pediatrics, the Chinese University of Hong Kong, Hong Kong, China

Liu JQ, Morton J, Miedzyblocki M, Lee TF, Bigam DL, Fok TF, Chen C, Lee SK, Davidge ST, Cheung PY. Sodium tanshinone IIA sulfonate increased intestinal hemodynamics without systemic circulatory changes in healthy newborn piglets. *Am J Physiol Heart Circ Physiol* 297: H1217–H1224, 2009. First published July 17, 2009; doi:10.1152/ajpheart.00477.2009.—In traditional Chinese medicine, tanshinone IIA is a lipid-soluble component of Danshen that has been widely used for various cardiovascular and cerebrovascular disorders, including neonatal asphyxia. Despite promising effects, little is known regarding the hemodynamic effects of tanshinone IIA in newborn subjects. To examine the dose-response effects of sodium tanshinone IIA sulfonate (STS) on systemic and regional hemodynamics and oxygen transport, 12 newborn piglets were anesthetized and acutely instrumented for the placement of femoral arterial and venous, pulmonary arterial catheters to measure mean arterial, central venous, and pulmonary arterial pressures, respectively. The blood flow at the common carotid, renal, pulmonary, and superior mesenteric (SMA) arteries were continuously monitored after treating the piglets with either STS (0.1–30 mg/kg iv) or saline treatment ($n = 6$/group). To further delineate the underlying mechanisms for vasorelaxant effects of STS, in vitro vascular myography was carried out to compare its effect on rat mesenteric and carotid arteries ($n = 4$–5/group). STS dose-dependently increased the SMA blood flow and the corresponding oxygen delivery with no significant effect on systemic and pulmonary, carotid and renal hemodynamic parameters. In vitro studies also demonstrated that STS selectively dilated rat mesenteric but not carotid arteries. Vasodilation in mesenteric arteries was inhibited by apamin and TRAM-34 (calcium-activated potassium channel inhibitors) but not by meclofenamate (cyclooxygenase inhibitor) or *N*-nitro-L-arginine methyl ester hydrochloride (nitric oxide synthase inhibitor). In summary, without significant hemodynamic effects on newborn piglets, intravenous infusion of STS selectively increased mesenteric perfusion in a dose-dependent manner, possibly via an endothelium-derived hyperpolarizing factor vasodilating pathway.

N-nitro-L-arginine methyl ester hydrochloride; common carotid artery

DANSHEN IS THE DRIED ROOT of *Salvia miltiorrhiza* (Fam. *Labiatae*) and is commonly used in traditional Chinese medicine for the treatment of various cardiovascular and cerebrovascular diseases, including myocardial infarct, angina pectoris, coronary heart disease, and cerebral stroke (6, 11, 35). Among the

ingredients, tanshinone IIA is the most abundant and well-studied lipophilic compound of Danshen (3). In regard to its cardiovascular effects, tanshinone IIA has been shown to cause coronary vasodilatation, reduce myocardial infarct size (21, 28), cardiomyocyte apoptosis (9), and hypertrophy (32), as well as to inhibit platelet aggregation (13). In critically ill neonates such as those with asphyxia, there is increasing evidence that ischemia-reperfusion injury contributes to some clinico-pathological complications, including shock, hypoxic-ischemic encephalopathy (HIE), and necrotizing enterocolitis (NEC) (23, 26, 27). Indeed, the pathogenesis of HIE and NEC has been suggested to be related to regional ischemia, and improving cerebral and mesenteric perfusion during recovery may be an effective therapeutic approach in the management of asphyxiated neonates (4, 23). Thus tanshinone IIA may have a therapeutic potential for asphyxiated neonates, including the possible improvement of systemic and regional perfusion. However, there is a lack of knowledge regarding the hemodynamic effects of tanshinone IIA and/or its derivatives in newborn subjects.

To address the potential for clinical use, it is essential to understand the dose-response of tanshinone IIA in newborn subjects. In view of the vascular effect of tanshinones, we aimed to investigate the dose-response of tanshinone IIA in systemic, pulmonary, and regional circulations in acutely instrumented newborn piglets. This information may help to determine if tanshinone IIA is a potential therapy that can specifically target one vascular bed without systemic effects. Furthermore, we used in vitro myography to investigate vascular bed specificity and to determine potential mechanisms of tanshinone IIA-induced vasodilation.

MATERIALS AND METHODS

All experiments were conducted in accordance with the guidelines of Canadian Council of Animal Care (2000) and were approved by the Animal Care and Use Committee, University of Alberta.

Materials. Twelve mixed-breed piglets (1–4 days of age, weighing 1.6–2.3 kg) were used for in vivo hemodynamic study. Spague-Dawley rats (3–4 mo of age, weighing 400–600 g) were used for in vitro vascular myography. Sodium tanshinone IIA sulfonate (STS, molecular formula $C_{19}H_{17}NaO_6S$, mol wt 396.39, purity 98%), a water-soluble derivative of Danshen, was purchased from Topharman Shanghai. The STS solution was freshly prepared daily with sterilized water before use. The pH of STS solution was 3.8 ± 0.1.

Animal preparation. Spontaneously breathing piglets were anesthetized initially via inhaled isoflurane (2–3%). Inhalational anesthe-

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Table 1. *Effect of STS on arterial blood gas*

	STS, mg/kg							P (STS vs. control)
	0	0.1	0.3	1	3	10	30	
Time (min):	Baseline	30	60	90	120	150	180	
				pH				0.28
STS	7.38±0.02	7.42±0.01	7.42±0.01	7.42±0.02	7.42±0.02	7.41±0.02	7.35±0.01	
Control	7.41±0.01	7.42±0.02	7.42±0.02	7.42±0.02	7.43±0.01	7.43±0.02	7.43±0.02	
				Pa_{CO_2}, *mmHg*				0.40
STS	40±2	36±1	36±1	37±2	38±1	40±1	46±1	
Control	39±1	38±1	38±0	38±1	38±1	35±2	38±1	
				Pa_{O_2}, *mmHg*				0.63
STS	68±2	70±3	67±2	64±3	66±2	63±2	57±1	
Control	64±1	68±3	62±1	65±4	67±6	67±7	62±3	
				HCO_3^-, *mmol/l*				0.64
STS	23±1	22±1	23±1	24±1	24±1	25±1	25±1	
Control	24±1	24±1	24±1	24±1	25±1	23±2	25±1	

Values are means ± SE. STS, sodium tanshinone IIA sulfonate. All $P > 0.05$, STS vs. control (2-way ANOVA).

sia was then switched to intravenous fentanyl (0.005–0.03 mg·kg^{-1}·h^{-1}), midazolam (0.1–0.2 mg·kg^{-1}·h^{-1}), and pancuronium (0.05–0.1 mg·kg^{-1}·h^{-1}) when the animal was mechanically ventilated through tracheostomy by a Sechrist infant ventilator (model IV-100; Sechrist Industries, Anaheim, CA) with pressures of 20/4 cmH$_2$O at rates of 15–20 breaths/min. Fractional inspired oxygen concentration was maintained between 0.21 and 0.25 to provide arterial oxygen saturation of 90–100%. Oxygen saturation was continuously monitored with a pulse oximeter (Nellcor, Hayward, CA). All medications and fluids were administered via the femoral venous catheter, and maintenance fluid consisted of 5% dextrose-saline at 20 ml·kg^{-1}·h^{-1}. The piglet's temperature was maintained at 38.5–39.5°C using an overhead warmer and a heating pad.

Surgical instrumentation. The surgical procedure was similar to that described previously (1). Under anesthesia, piglets were catheterized with Argyle catheters (5 Fr.; Sherwood Medical, St. Louis,

MO) via the right femoral artery and vein to the level of infrarenal aorta and right atrium, respectively. The arterial catheter was used to monitor mean arterial pressure (MAP), whereas the venous catheter was used for central venous pressure (CVP) with a 78833B monitor (Hewlett Packard). After tracheotomy was established through a midline neck incision, the left common carotid artery was isolated and circled by a 2-mm transit time ultrasound flow probe (2SS; Transonic Systems, Ithica, NY). After a left anterior thoracotomy with pericardium exposed, a 20-gauge angiocatheter (Insite) was inserted in the root of the pulmonary artery for the measurement of mean pulmonary arterial pressure (PAP). A 6-mm Transonic flow probe (6SB) was placed at the root of the main pulmonary artery to measure the blood flow, which was used as a surrogate of cardiac output. The superior mesenteric (SMA) and renal arteries were isolated and encircled with a 3-mm (3SB) and a 2-mm (2SB) Transonic flow probe via a left flank incision, respectively. All incisions were covered to minimize evap-

Table 2. *Effects of STS on systemic and pulmonary haemodynamics*

	STS, mg/kg							P (STS vs. control)
	0	0.1	0.3	1	3	10	30	
Time (min):	Baseline	30	60	90	120	150	180	
				Heart rate, beats/min				0.35
STS	182±8	185±9	188±14	190±12	191±10	185±7	193±6	
Control	164±10	165±12	163±14	166±13	177±14	183±15	191±17	
				Cardiac index, ml·min^{-1}·kg^{-1}				0.79
STS	166±17	168±18	167±19	170±17	175±17	181±15	191±15	
Control	173±14	170±16	170±15	180±14	185±14	192±14	192±16	
				Stroke volume index, ml/kg				0.31
STS	0.91±0.08	0.91±0.08	0.90±0.08	0.90±0.09	0.93±0.09	0.98±0.07	1.00±0.09	
Control	1.09±0.12	1.08±0.14	1.09±0.14	1.13±0.13	1.09±0.12	1.05±0.11	1.04±0.10	
				Systemic vascular resistance index, mmHg·ml^{-1}·min^{-1}·kg^{-1}				0.32
STS	0.44±0.04	0.42±0.04	0.42±0.04	0.39±0.03	0.35±0.03*	0.32±0.02*	0.29±0.02*	
Control	0.40±0.03	0.39±0.059	0.39±0.04	0.35±0.03	0.34±0.02*	0.31±0.02*	0.32±0.02*	
				Estimated pulmonary vascular resistance index, mmHg·ml^{-1}·min^{-1}·kg^{-1}				0.74
STS	0.17±0.03	0.17±0.03	0.17±0.03	0.17±0.03	0.18±0.03	0.18±0.02	0.18±0.02	
Control	0.16±0.03	0.17±0.04	0.17±0.04	0.16±0.03	0.15±0.03	0.15±0.03	0.16±0.02	

Values are means ± SE. *$P < 0.05$ vs. baseline (1-way ANOVA). All $P > 0.05$, STS vs. control (2-way ANOVA).

orative heat loss. Heart rate, cardiac output, MAP, PAP, CVP and common carotid artery (CCA), pulmonary arterial, SMA and renal artery (RA) flows were continuously monitored, and all data were recorded by a DT 2801-A analog-to-digital converter board (Data Translation, Ontario, Canada). For each hemodynamic parameter, an average of a 2-min range at the end of administration of each dosage during the experimental period was taken.

Experimental protocol. After 60 min of stabilization, piglets were randomized into either a control or a STS-treated group ($n = 6$/group). Based on dosages reported in the literature (1–10 mg/kg iv and 2–60 mg/kg ip in rats) (2), an intravenous dosage range of 0.1–30 mg/kg was selected in the present study. The doses of STS were given in an accumulated dose manner (0.1, 0.3, 1, 3, 10, or 30 mg/kg) stepping up every 30 min for a total duration of 3 h. Because the plasma concentration of STS peaked within 30 min after intravenous injection in rats (2), the physiological changes after any given dose were observed for 30 min. The total injection volume of STS was 10 ml; 5 ml (50% of the dose) were given over 5 min as a bolus, whereas another 5 ml (the remaining 50% dose) were infused over 25 min. The control group received the same volume of 0.9% normal saline (pH 5.5). At the end of the study, piglets were killed with an overdose of pentobarbital sodium (100 mg/kg iv).

Hemodynamic measurements and oxygen transport. The pulmonary arterial, CCA, SMA and RA blood flows were indexed with piglet weight and expressed as cardiac index (CI), common carotid artery flow index, superior mesenteric artery flow index, and renal artery flow index (units: $ml \cdot kg^{-1} \cdot min^{-1}$), respectively. The hemodynamic variables were calculated as follows:

Systemic oxygen delivery (ml $O_2 \cdot kg^{-1} \cdot min^{-1}$)
$$= CI \times Sa_{O_2} \times 1.34 \times [Hb], \quad (1)$$

Systemic oxygen consumption (ml $O_2 \cdot kg^{-1} \cdot min^{-1}$)
$$= CI \times (Sa_{O_2} - Sv_{O_2}) \times 1.34 \times [Hb]. \quad (2)$$

Systemic oxygen extraction ratio (%)
$$= [(Sa_{O_2} - Sv_{O_2})/Sa_{O_2}] \times 100\%. \quad (3)$$

Simultaneous blood samples were drawn for evaluation of blood gases, arterial and mixed venous oxygen saturation by ABL500 and OSM 3 Hemoximeter (Radiometer, Copenhagen, Denmark) at the same time points. Based on the blood gas saturation [arterial saturation (Sa_{O_2}); venous saturation (Sv_{O_2})] and hemoglobin (Hb) concentration, the oxygen delivery and consumption were calculated as follows.

Carotid arterial oxygen delivery (ml $O_2 \cdot kg^{-1} \cdot min^{-1}$)
$$= CCA \text{ flow index} \times Sa_{O_2} \times 1.34 \times [Hb] \quad (4)$$

where [Hb] is Hb concentration.

Superior mesenteric arterial oxygen delivery (ml $O_2 \cdot kg^{-1} \cdot min^{-1}$)
$$= SMA \text{ flow index} \times Sa_{O_2} \times 1.34 \times [Hb] \quad (5)$$

Renal arterial oxygen delivery (ml $O_2 \cdot kg^{-1} \cdot min^{-1}$)
$$= RA \text{ flow index} \times Sa_{O_2} \times 1.34 \times [Hb] \quad (6)$$

Stroke volume index $(ml \cdot kg^{-1} \cdot beat^{-1}) = CI/heart rate \quad (7)$
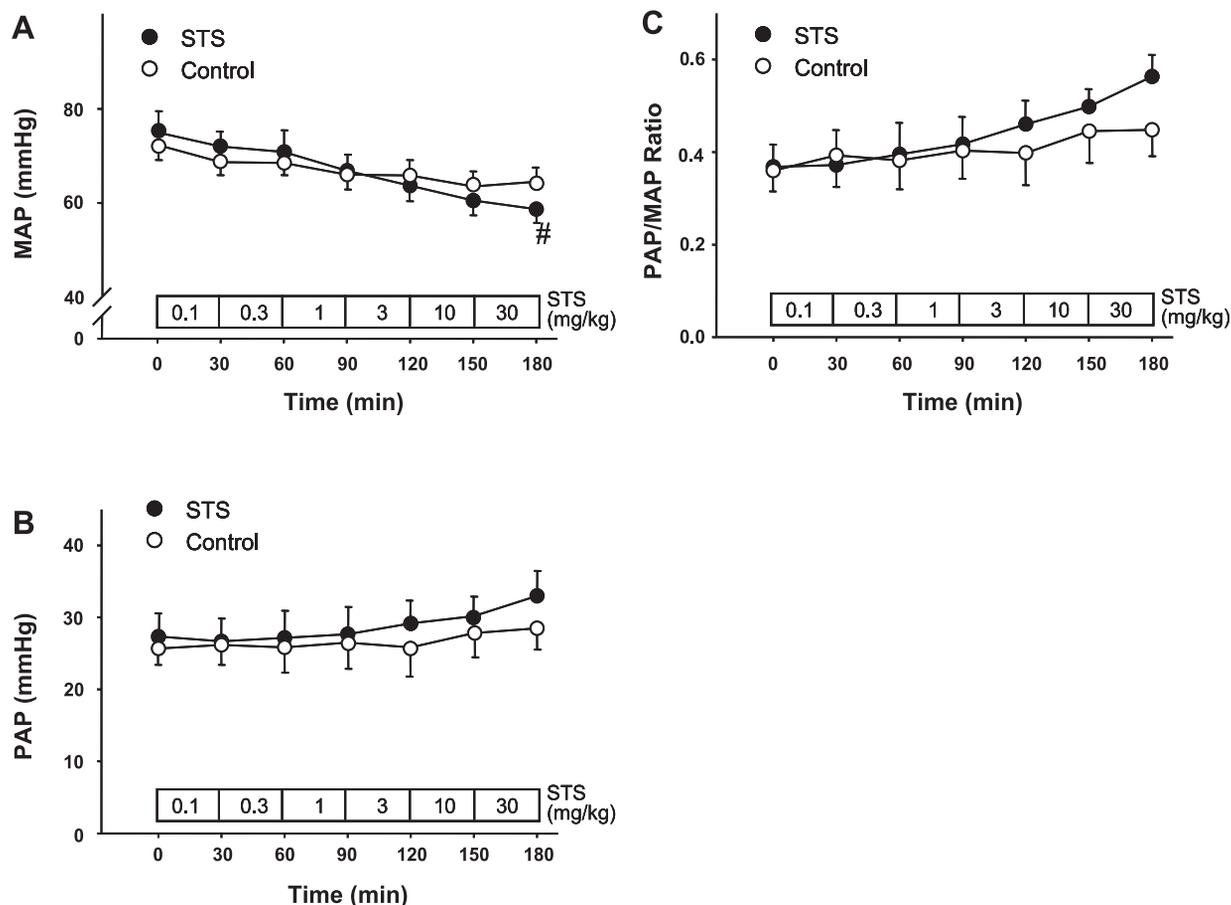

Fig. 1. Cumulative dose effect of sodium tanshinone IIA sulfonate [STS (●), $n = 6$] on mean arterial pressure (MAP; *A*), mean pulmonary arterial pressure (PAP; *B*), and the PAP-to-MAP ratio (*C*) compared with saline [control (○)] ($P > 0.05$, 2-way ANOVA). #$P < 0.05$ vs. baseline (1-way ANOVA).

Systemic vascular resistance index

$$(\text{mmHg} \cdot \text{ml}^{-1} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}) = (\text{MAP} - \text{CVP})/\text{CI} \qquad (8)$$

Pulmonary vascular resistance index

$$(\text{mmHg} \cdot \text{ml}^{-1} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}) \text{ was estimated by PAP/CI} \qquad (9)$$

In vitro vascular myography. Small mesenteric (129 ± 6 μm) and carotid (675 ± 23 μm) arteries were isolated from young male Sprague-Dawley rats. The arteries were mounted on two 25-μm wires attached to a wire myograph (DMT; Copenhagen, SV, Denmark) to allow isometric tension recordings. Vessels were normalized to determine their optimal resting tension.

Following a 30-min equilibration period, vessels were exposed two times to a single concentration of phenylephrine (10 μM) followed by a single concentration of methylcholine (3 μM) to check functional endothelial and smooth muscle integrity. A concentration-response curve (CCRC) to either phenylephrine (mesenteric arteries) or U-46619 (carotid arteries) was performed to determine the EC_{80} for each vasoconstrictor. Vasoconstrictors were chosen based on our previous studies demonstrating arterial bed specific responses to adrenergic agonists. Pilot data using STS showed vasodilation was not dependent on the vasoconstrictor agonist used. To investigate vascular responses to STS (1–300 μM), a CCRC was performed following preconstriction with either phenylephrine or U-46619. To determine if vasodilator responses to STS were mediated via endothelium-derived

relaxing factors, CCRCs were performed in the absence or presence of the potassium channel inhibitors apamin (small-conductance, calcium-activated potassium channels, 100 nM) and TRAM-34 (intermediate-conductance, calcium-activated potassium channels, 10 μM) or the nitric oxide synthase inhibitor *N*-nitro-L-arginine methyl ester hydrochloride (L-NAME, 100 μM) or the cyclooxygenase inhibitor meclofenamate (1 μM).

Statistical analysis. Results are expressed as means ± SE. Two-way and one-way repeated-measures analysis of variance were used to compare differences in hemodynamic variables between groups and between different doses of STS and baseline, respectively. Friedman's test was used to compare the differences for nonparametric variables. Student-Newman-Keul's and Dunn's test were used for post hoc analysis as appropriate. Regarding the in vitro vascular myography, the data are expressed as means ± SE of the negative log of the effective concentration (mol/l) that will give 50% of the maximum response (pEC_{50}) or the maximum response (E_{max}). The significance of the difference between groups was determined by two-way ANOVA with Bonferroni's posttest. Significance was defined as $P < 0.05$.

RESULTS

Comparing the control and STS-treated piglet groups, there were no significant differences in the age (2.6 ± 0.7 vs. 2.8 ±
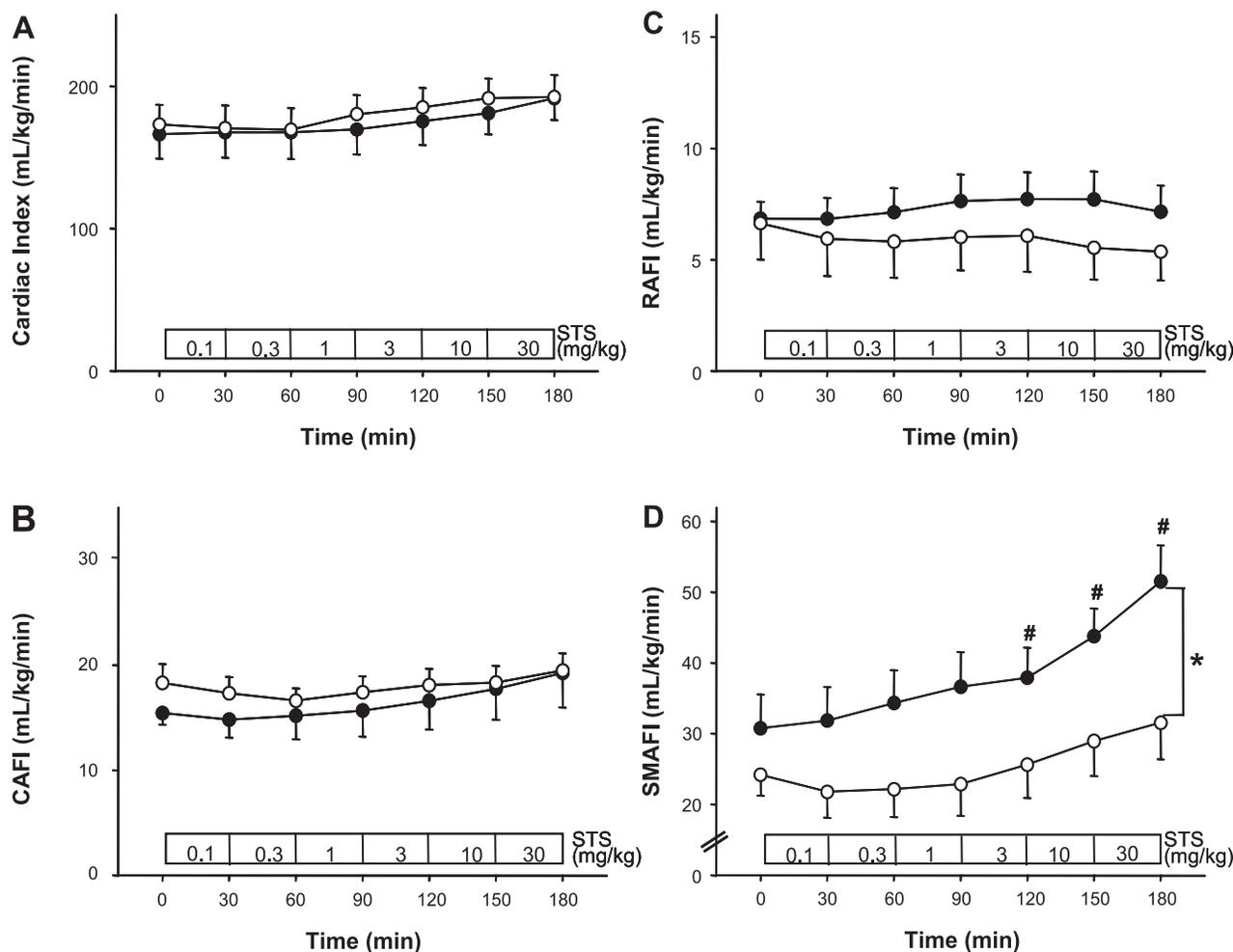

Fig. 2. Cumulative dose effect of STS (●, $n = 6$) on cardiac index (*A*), common carotid artery flow index (CAFI; *B*), renal artery flow index (RAFI; *C*), and (*D*) superior mesenteric artery flow index (SMAFI; *D*) index compared with saline [control (○)]. $P < 0.05$ vs. controls (2-way ANOVA; *) and vs. baseline (1-way ANOVA; #).

0.4 days, respectively), weight (1.9 ± 0.1 vs. 2.2 ± 0.1 kg, respectively), blood gas, and hemodynamic measurements at baseline. The physiological and hemodynamic parameters of control piglets did not change significantly throughout the experimental period. The administration of STS did not affect the arterial blood gas measurements ($P > 0.05$ vs. baseline and the corresponding control values) (Table 1).

STS does not cause significant systemic and pulmonary hemodynamic effects. Intravenous administration of STS did not cause any significant changes in heart rate, CI, stroke volume, PAP, and estimated pulmonary vascular resistance index (VRI; Table 2 and Fig. 1). No episode of arrhythmia was identified during the administration of STS. STS at 30 mg/kg decreased MAP compared with baseline ($P < 0.05$), leading to a higher PAP-to-MAP ratio; however, these changes were not significantly different from the corresponding control values. The systemic VRI of piglets treated with STS at 3, 10, and 30 mg/kg and those corresponding systemic VRI of controls were lower than the respective baseline (both $P < 0.05$), with no difference between the two groups ($P > 0.05$).

STS selectively increases mesenteric hemodynamic parameters. SMA flow increased in a dose-dependent fashion in STS-treated piglets ($P < 0.05$) and became significantly higher

than the baseline value at 3, 10, and 30 mg/kg (38 ± 4, 44 ± 4, and 52 ± 5 vs. 31 ± 5 ml·kg^{-1}·min^{-1} of baseline, respectively; $P < 0.05$) (Fig. 2). There were no significant changes in common carotid and renal arterial flows with STS (0.1–30 mg/kg) administration.

Effects of STS on systemic and regional oxygen transport. Corresponding to the changes in SMA flow, the SMA oxygen delivery was significantly increased when STS at 3, 10, and 30 mg/kg was given (0.43 ± 0.06, 0.46 ± 0.08, and 0.49 ± 0.07 vs. 0.31 ± 0.01 ml O$_2$·min^{-1}·kg^{-1} of baseline, respectively) (Fig. 3). STS did not cause any significant change in systemic, common carotid, and renal arterial oxygen delivery. There were no significant differences in systemic oxygen consumption and extraction ratio in the STS-treated group compared with the respective baseline and corresponding control values (data not shown).

In vitro vascular myography in mesenteric and carotid arteries. STS caused a concentration-dependent vasodilation in small mesenteric arteries (pEC$_{50}$ = 4.4 ± 0.04, E$_{max}$ = 99.1 ± 0.9% relaxation) but not in carotid arteries (Fig. 4). Vasodilation in mesenteric arteries was inhibited by a combination of potassium channel blockers (apamin, 100 nM and TRAM-34, 10 μM) causing a rightward shift of the STS response (pEC$_{50}$ = 4.0 ± 0.06,
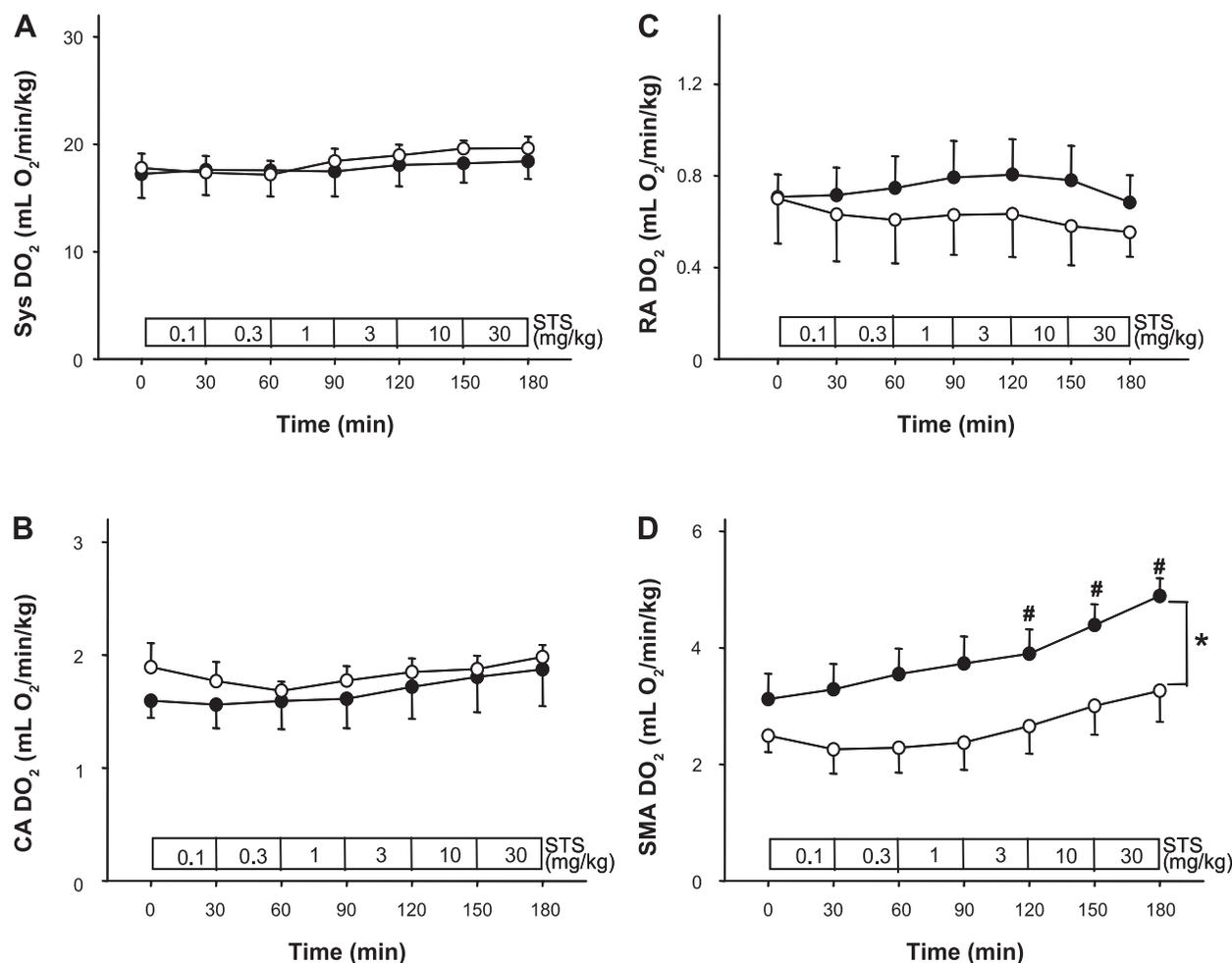

Fig. 3. Cumulative dose effect of STS (●, $n = 6$) on systemic oxygen delivery (Sys DO$_2$; *A*), common carotid artery oxygen delivery (CA DO$_2$; *B*), renal artery oxygen delivery (RA DO$_2$; *C*), and superior mesenteric artery oxygen delivery (SMA DO$_2$; *D*), compared with saline (control, ○). $P < 0.05$ vs. controls (2-way ANOVA; *) and vs. baseline (1-way ANOVA; #).
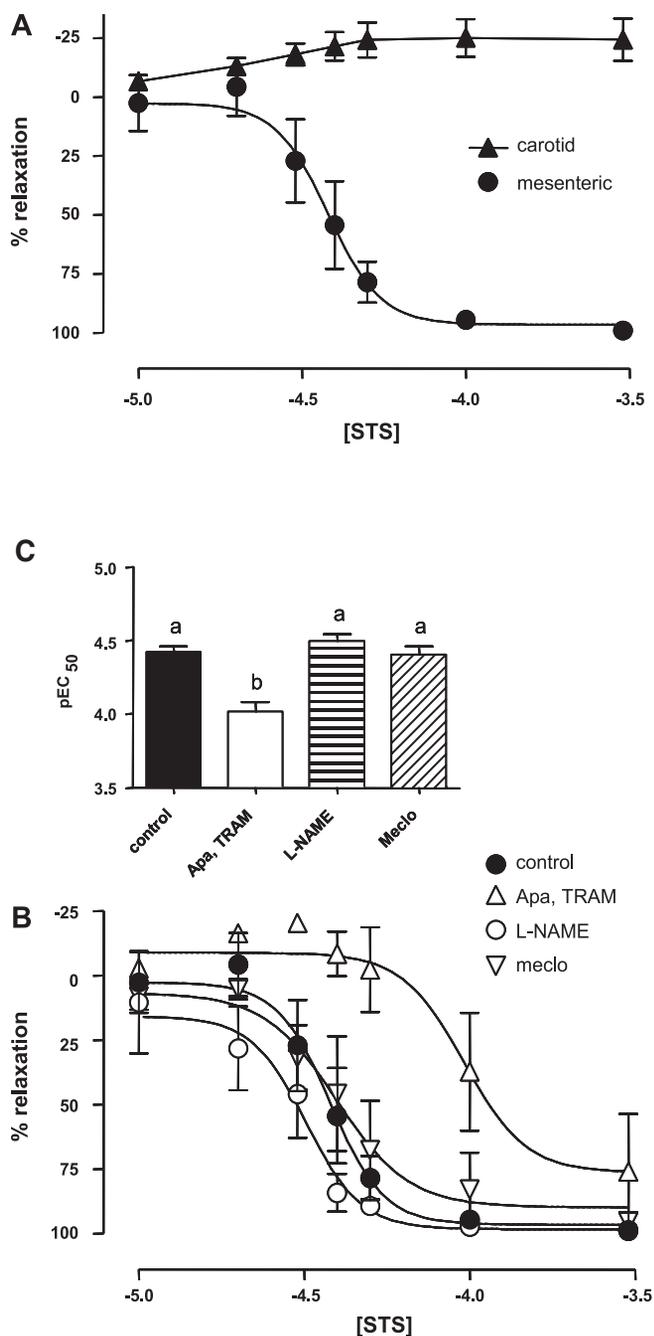
Fig. 4. *A*: vasodilation in response to STS (1–300 μM) in mesenteric (●) but not carotid (▲) arteries. *B*: vasodilation to STS in mesenteric arteries (●) is inhibited by a combination of apamin (Apa, small-conductance calcium-activated potassium channel inhibitor, 100 nM) and TRAM-34 [TRAM, intermediate-conductance calcium-activated potassium channel inhibitor, 10 μM (△)] but not by *N*-nitro-L-arginine methyl ester hydrochloride [L-NAME (○), nonspecific nitric oxide synthase inhibitor, 100 μM] or meclofenamate [meclo (▽), nonspecific cyclooxygenase inhibitor, 1 μM]. *C*: the negative log of the effective concentration (mol/l) that will give 50% of the maximum response (pEC_{50}) is significantly decreased in the presence of Apa and TRAM compared with all other groups, $P < 0.001$ (ANOVA). Different letters denote significant difference ($P < 0.05$) from each other.

$P < 0.001$; Fig. 4, *B* and *C*). Neither the nitric oxide synthase inhibitor L-NAME ($pEC_{50} = 4.5 \pm 0.05$) nor the cyclooxygenase inhibitor meclofenamate ($pEC_{50} = 4.4 \pm 0.06$) inhibited mesenteric artery responses to STS.

DISCUSSION

To the best of our knowledge, this is the first study to investigate the systemic, pulmonary, and regional hemodynamic effects of intravenous administration of STS in healthy newborn subjects. Even though Danshen has been widely used in traditional Chinese medicine for various cardiovascular diseases (6, 11, 35), little is known about its hemodynamic effects, which is important in translational research. In this study, we demonstrated that intravenous STS dose-dependently increased SMA blood flow and oxygen delivery, without significant systemic, pulmonary, carotid, and renal hemodynamic effects except at the highest studied dosage of 30 mg/kg in newborn piglets. Interestingly, the mechanism for vasorelaxation does not rely on nitric oxide, and this could be important in states of oxidative stress where nitric oxide could be detrimental.

In part, similar to a previous report that purified tanshinones from Danshen caused in vitro vasodilatation of mesenteric, renal, and femoral arteries in adult rats and rabbits (20), we also observed that STS dose-dependently increased SMA blood flow in newborn piglets. However, the vasodilatory effect of STS appears to be tissue specific, since no significant vascular changes in the carotid and renal circulations were observed after receiving the similar dosage range of STS. Corresponding to dose-dependent changes in blood flow, SMA oxygen delivery was increased with STS administration.

NEC is the leading cause of death and long-term disability from gastrointestinal disease in preterm infants and is characterized by acute and chronic intestinal inflammation that may lead to systemic sepsis and multisystem organ failure (19, 22). Because ischemia is one of the pathophysiological factors for NEC in asphyxiated term neonates (12, 23), the improvement of mesenteric perfusion with STS infusion could be beneficial to neonates who are at risk for ischemic intestinal injury. By reducing leukocyte adhesion and scavenging oxygen-free radicals, it has also been shown recently that injection of Danshen extract can improve endotoxin-induced microcirculatory disturbance in rat mesentery (11). In view of the fact that minimal side effects have been observed in the current study, further investigations are thus worthwhile to examine this possibility of employing STS as a pharmacological therapy for treating or preventing NEC in critically ill neonates.

To further delineate the underlying mechanisms for vasorelaxant effects of STS, in vitro vascular myography was also carried out to compare its effect on rat mesenteric (resistance) and carotid (conduit) arteries. Despite differences in species (pigs vs. rats) and age (newborn vs. adult), STS also selectively caused a concentration-dependent vasodilation in SMA, but not carotid, artery. Although vasodilators such as acetylcholine are commonly mediated by a combination of nitric oxide, prostaglandins, and endothelium-derived hyperpolarizing factor, STS responses were not mediated by either nitric oxide or prostaglandins. Our findings further suggest that either STS is acting through a very specific, potassium channel-dependent pathway or through an as yet undetermined additional mechanism. Indeed, we believe that STS is likely causing a vasodilator response via the activation of endothelial potassium channels. STS has previously been shown to activate calcium-activated potassium channels in coronary arteries (33). The inhibitors of endothelial intermediate- and small-conductance

calcium-activated potassium channels (TRAM-34 and apamin, respectively) used in this study are commonly accepted to be synonymous with inhibition of endothelium-derived hyperpolarizing factor-mediated vasodilation. Activation of these channels causes hyperpolarization of the endothelial cell membrane, which is then transmitted to the smooth muscle cell to cause relaxation. Similarly, it has been shown that the vasodilatory effect of Danshen extract on rat isolated femoral artery was only blocked by a nonselective potassium channel blocker, but not by various receptor blockers or adenylyl and guanylyl cyclase inhibitors (18). Contrary to our observation that endogenous nitric oxide may not be involved in STS-induced vasorelaxation, several in vitro (14, 17) and in vivo (15) studies have shown that Danshen and its extract caused vasodilation mainly by increasing nitric oxide production in chronic studies. Other than the differences in extract components used in the studies, the discrepancy may also be due to tissue specificity. It has been shown from the same research group that the vasodilatory effect of Danshen extract on knee joint blood vessel (17), but not femoral artery (18), was blocked by nitric oxide synthase inhibitor. Other potential mechanisms of the effect of Tanshinone IIA on blood flow include the regulation of phospholipase A_2 (31) and the activation of estrogen receptors (8).

In contrast to a previous study in albino rats and rabbits showing that Danshen significantly decreased systemic blood pressure (20), we observed a decrease in MAP only after treating the piglets with the highest dose of STS (30 mg/kg). However, it is worth noting that STS has recently been shown to reduce MAP only in hypertensive, but not healthy sham-operated, hamsters (15). As indicated by the corresponding decreases in systemic VRI, it is most likely that the decrease in blood pressure resulted from systemic vasodilatation (20). In view of the fact that initial hyperemia following hypoxia or ischemia represents a prerequisite to neuronal functional recovery (10), the vasodilating effect of STS could be beneficial to improve recovery from asphyxia.

The systemic vascular effect of STS may have interesting implications. As shown in Table 2, STS caused a dose-related increase in the PAP-to-MAP ratio, which was significantly higher than the baseline value at 30 mg/kg, in the absence of any significant changes in PAP. We do not know the significance of this increase in the ratio by ~50% from the baseline. This elevation might not be clinically relevant, since the ratio was still below 1.0: an equivalent or suprasystemic ratio suggests a reversal of left-to-right shunt across a patent ductus arteriosus in the transitional pulmonary circulation of sick neonates (1, 25). Indeed, the nonsignificant elevated Pa_{CO_2} and decreased Pa_{O_2}, which remained within the clinically acceptable ranges, may support a possible effect of an increased PAP-to-MAP ratio. Taken together, we should exercise caution on the decreased systemic VRI and MAP, and increased PAP-to-MAP ratio with the highest dosage of STS (30 mg/kg) compared with the respective baseline. Although there were no significant differences from the corresponding control measurements, the intravenous administration of high-dose STS (>10 mg/kg) in neonates should be proceeded with care because of the potential adverse vascular effect on systemic and pulmonary circulations. Nevertheless, similar to the lack of significant effect of STS on heart rate in healthy subjects, we did not observe any significant effect of STS on heart rate, CI,

and stroke volume during the experimental period in these healthy newborn piglets.

In summary, intravenous administration of STS may be potentially beneficial in improving intestinal perfusion, which is likely dependent on the endothelium-derived hyperpolarizing factor but not nitric oxide pathway. Intravenous STS does not affect overall hemodynamics on newborn piglets at a dose range of 0.1–1 mg/kg. Further studies are required to demonstrate the effectiveness of STS as a therapeutic agent for NEC in newborn subjects.

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