β₃-Adrenoceptor Antagonist SR59230A Attenuates the Imbalance of Systemic and Myocardial Oxygen Transport Induced by Dopamine in Newborn Lambs

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Abstract

Background: In neonates, the increase in O₂-delivery (DO₂) by dopamine is offset by a greater increase in O₂-consumption (VO₂). This has been attributed to β₃-adrenergic receptors in neonatal brown fat tissue. β₁ receptors in the heart have negative inotropic properties. We evaluated the effects of SR59230A, a β₃-antagonist, on the balance of systemic and myocardial O₂-transport in newborn lambs treated with dopamine.

Methods: Lambs (2–5 days old, n = 12) were anesthetized and mechanically ventilated. Heart rate (HR) and rectal temperature were monitored. VO₂ was measured by respiratory mass spectrometry and cardiac output (CO) by a pulmonary artery transonic flowmeter. Arterial, jugular bulb venous and coronary sinus blood gases and lactate were measured to calculate DO₂, O₂ extraction ratio (ERO₂), myocardial O₂ and lactate extraction ratios (mERO₂, mERlac). After baseline measurements, lambs were randomized to receive SR59230A at 5 mg/kg iv (SRG) or placebo. Both groups received incremental doses of a dopamine infusion (0–5–10–15–20 mcg/kg/min) every 15 min. Measurements were repeated at the end of each dose.

Results: After SR59230A infusion, CO and HR trended to decrease (P = 0.06), but no significant changes occurred in other parameters. Over the incremental doses of dopamine, temperature increased in both groups (P < 0.0001) but to a lesser degree in SRG (P = 0.004). CO and HR increased (P = 0.005 and 0.04) and similarly in both groups (P > 0.1). DO₂ trended to a small increase (P = 0.08). VO₂ increased in both groups (P < 0.0001) but to a lesser degree in SRG (P < 0.0001). As a result, ERO₂ increased in both groups (P < 0.0001), but to a lesser degree in SRG (P < 0.0001). mERO₂ was lower in SRG (P = 0.01) with a faster increase (P < 0.0001). mERlac was higher in SRG (P = 0.06) with a faster decrease (P = 0.04).

Conclusion: Although SR59230A tends to induce an initial drop in CO, it significantly attenuates the rise in VO₂ and hence the imbalance of systemic and myocardial O₂ transport induced by dopamine at higher doses. Studies are warranted to examine the effect of SR59230A in cases of cardiac dysfunction and increased VO₂, observed after cardiac surgery.

Keywords: β₃-adrenoreceptors, SR59230A, newborn lambs, oxygen consumption
Introduction
Dopamine, a precursor in the endogenous synthesis of norepinephrine, is the most commonly used medication to provide cardiovascular support in critically ill patients with low cardiac output state, via the stimulation of β1 and β2 adrenergic receptors. Often, these patients have impaired balance of oxygen transport and poor tissue perfusion. Therefore, in addition to improved cardiovascular state, the major goal of therapy in these patients is to improve the balance of systemic oxygen transport and tissue oxygenation by increasing systemic O₂ delivery (DO₂) relative to changes in systemic O₂ consumption (VO₂). In adult humans and animal experiments, the increase in DO₂ induced by catecholamines is about 10-fold of the increase in VO₂. However, in neonates, in contrast to that observed in adults and older children, dopamine worsens the balance of systemic oxygen transport by a greater increase in VO₂ than DO₂ as shown after cardiopulmonary bypass surgery. In neonatal lambs, dobutamine, a synthetic analog of dopamine, increases VO₂ by 7–12 fold compared to older lambs, with a similar increase in DO₂. The substantial increase in VO₂ has been attributed to the abundant brown adipose tissue in neonates, which contains plentiful α, β₁, and β₃ adrenergic receptors. Stimulation of these receptors by catecholamines leads to non-shivering thermogenesis and a substantial increase in VO₂. β₃-adrenergic receptor was discovered in 1970s and 1980s from pharmacological studies of rat adipocyte function, and is now known to be richly present in brown adipose tissue controlling of thermogenesis and energy balance. It has been shown that dopamine exerts a thermogenic effect on the brown adipose tissue and increases VO₂ in rats. Selective β₃-adrenoceptor antagonist, SR59230A, has been reported to down-regulate uncoupling protein-1 and norepinephrine induced cAMP accumulation, thus reduce thermogenesis in brown fat tissue in lambs. In addition, it has been found that β₃-adrenergic receptors in the heart have negative inotropic properties. This has led to research investigating potential therapeutic applications to enhance cardiac contractility by blocking β₃-adrenergic receptors in heart failure.

Therefore, we hypothesized that SR59230A may attenuate the increase in VO₂ induced by dopamine, and maintain the inotropic effect, thus cardiac output (CO) and DO₂, thereby improving the balance of systemic and myocardial oxygen transport in neonatal lambs.

Methods and Materials
Animal preparation
All experiments were conducted in accordance with guidelines and by approval of the Animal Care and Use Committee (Health Science), University of Alberta. Twelve mixed-breed newborn lambs 1 to 5 days of age weighing 2.5 to 5.0 kg were obtained from a local farm. Anesthesia was induced with isoflurane (2%–3%). After induction, isoflurane inhalation was ceased, and anesthesia was maintained with continuous i.v. infusion of fentanyl (5–10 µg/kg/h), midazolam (0.1–0.2 mg/kg/h) and pancuronium (0.05–0.1 mg/kg/h). Animals were intubated with auffed endotracheal tube to prevent any air leaks, and ventilated with FiO₂ 0.3 and volume-cycled ventilation (SERVO VENTILATOR 300, Siemens Medical Systems, Solna, Sweden), a tidal volume of 10 mL/kg, rates of 20–30 breaths/min, and PEEP of 4 cm H₂O. Systemic arterial blood gases were measured (iStat, Abbott Laboratories. Abbott Park, IL USA) and ventilation was adjusted to maintain pH 7.35–7.45, PaO₂ 100–140 mmHg, and PaCO₂ at 35–40 mmHg, while base deficits >2 mmol/L were corrected with 22 mEq/L NaHCO₃. Blood that had been collected from an adult donor lamb was used for blood transfusion at a rate of 20 to 30 mL/h.

Left femoral arterial, superior vena cava, coronary sinus catheters were inserted to monitor pressures, blood gases and to administer fluids and medications. Heart rate was measured (Hewlett Packard, Palo Alto, CA). Maintenance fluids consisted of 5% dextrose-saline at 10 mL/kg/h. Temperature was monitored by a rectal thermometer, and maintained above 37 °C by a heating pad and warm blankets, which were kept constant during the experimental period. A sternotomy was performed and the ductus arteriosus was ligated. An 8–10 mm diameter ultrasonic perivascular flow probe (Transonics Systems, Ithaca, New York, USA)
was placed around the main pulmonary artery to continuously measure CO.

VO₂ was continuously measured using respiratory mass spectrometry (AMIS2000 Inovision A/S, Odense, Denmark). This is highly sensitive, accurate and rapid method to allow simultaneous measurements of multiple gas fractions, and has been described previously.¹⁷ Stroke volume was calculated as CO/heart rate. DO₂ was calculated as CO times arterial oxygen content (CaO₂). Oxygen extraction ratio (ERO₂) was calculated as VO₂/DO₂. All systemic hemodynamic and oxygen transport variables were indexed by weight as appropriate.

Myocardial oxygen and lactate extraction ratios (mERO₂, mERlac, respectively) were calculated using standard equations:

\[
\text{mEO}_2 = \frac{(\text{CaO}_2 - \text{CcsO}_2)}{\text{CaO}_2}
\]

\[
\text{mERlac} = \frac{(\text{arterial lactate concentration} - \text{coronary sinus lactate concentration})}{\text{arterial lactate concentration}}
\]

where CcsO₂ indicates coronary sinus oxygen content.

**Experimental protocol**

Lambs were randomized into two groups (n = 6 each) after a period of stabilization and baseline measurements of hemodynamics, oxygen transport and central temperature were obtained. Subsequently, the experimental (SR group) received an i.v. infusion of SR59230A at 5 mg/kg dissolved firstly in 0.5 mL DMSO, and then in 10 mL saline and an equal volume of the DMSO and saline, respectively, over 5 min. After 30 min, incremental dopamine i.v. infusions starting from 5 were increased to 10, 15, and 20 µg/kg/min sequentially every 15 minutes. Measurements were repeated before dopamine infusion and at the end of each dose of dopamine in both groups. At the end of the study, an overdose of phenobarbital was given for euthanasia.

**Statistical analysis**

Data are expressed as mean ± SD. Mixed linear regression for repeated measures was used to compare the variables before and after SR59230A or saline infusion between the two groups. It was also used to compare the different levels of the variables and their trends of changes between groups over the incremental increases of dopamine, with analyses of the effects of group (P<sub>group</sub>) and dose (P<sub>dose</sub>) and the interaction between dose and group (P<sub>dose*group</sub>). All data analysis was performed using SAS statistical software version 9.2 (SAS Institute Inc). A P value <0.05 indicates a statistical significance.

**Results**

There were no significant differences in age (mean 3 days old) and weight (4.0 ± 1.0 kg vs. 3.7 ± 0.6 kg) between the SR and Control groups, respectively.

**Changes of the variables before and after SR 59230A/saline infusion in the two groups (Table 1 and Fig. 1)**

All hemodynamic and oxygen transport variables were not significantly different at baseline between the two groups. Thirty minutes following SR59230A infusion, temperature and mean arterial pressure remained unchanged and were not different from those of Control group. Heart rate trended to decrease from 237 ± 33 beats/min to 202 ± 7 beats/min in SR group (P = 0.08). CO trended to decrease from 167 ± 31 mL/min/kg to 123 ± 8 mL/min/kg in SR group (P = 0.08). Stroke volume remained similar after SR59230A infusion within both groups. DO₂, VO₂, ERO₂, mERO₂ and mERlac did not change significantly in SR group. There were no significant changes in all the variables in Control group.

**Changes of the variables over the incremental infusion of dopamine in the two groups (Table 1 and Fig. 1)**

Over incremental doses of dopamine, temperature increased significantly in both groups (P<sub>dose</sub> < 0.0001); the rate of temperature increase was significantly less in SR group compared to Control group (P<sub>dose*group</sub> < 0.005). There was no significant change in mean arterial pressure in either SR or Control group. Heart rate significantly increased in both groups (P<sub>dose</sub> < 0.05) to a similar degree (P<sub>dose*group</sub> > 0.10). CO significantly increased in both groups (P<sub>dose</sub> < 0.005) to a similar degree (P<sub>dose*group</sub> > 0.10). There was no significant change in stroke volume in both groups. DO₂ trended to a small increase (P<sub>dose</sub> = 0.08) due to a small but significant decrease in hemoglobin in both groups (P<sub>dose</sub> = 0.04). VO₂ increased significantly in both groups (P<sub>dose</sub> < 0.0001); however, the rate of VO₂
The rate of overall increase in ERO₂ was significantly less in SR group compared to Control group ($P_{dose*group} < 0.0001$). As a result, ERO₂ increased significantly in both groups ($P_{dose} < 0.0001$). The rate of overall increase in ERO₂ was significantly less in SR group ($P_{dose*group} < 0.0001$), and it started to decrease at the incremental dose of dopamine from 10 to 20 μg/kg/min. mERO₂ did not increase significantly in both groups ($P > 0.10$). mERO₂ was significantly lower in SR group ($P_{group} = 0.01$), with faster increase in SR groups compared to Control group ($P < 0.0001$). mERlac decreased significantly in both groups ($P = 0.04$). mERlac levels trended to be higher ($P = 0.08$) with faster decrease over the doses of dopamine ($P = 0.04$).

**Discussion**

This study demonstrates that in newborn lambs, SR59230A, a selective β₁-adrenoreceptor antagonist, significantly attenuated the imbalance in systemic oxygen transport seen with incremental dopamine infusion. These increases in VO₂ levels were significantly attenuated by SR59230A, whilst the increases in DO₂ were not significantly different between the two groups. As a result, the balance of systemic and myocardial oxygen transport saw a significant increase with incremental dopamine infusion.
transport was improved, as indicated by the attenuated increase in ERO₂, lower mERO₂ and higher mERlac.

The goal of dopamine and other catecholamine therapy is to improve the balance of systemic oxygen transport by a greater increase in DO₂ relative to changes in VO₂. Both circulatory and metabolic stimulating effects by catecholamines share the same adrenergic signaling mechanisms, mainly, β₁ and β₂ and α-adrenergic receptors. If the circulatory responses to adrenergic stimulation are reduced, or the metabolic response enhanced, the beneficial effects of catecholamines might be abrogated. Such is the case in neonatal subjects particularly those with myocardial injury and increased VO₂ as seen after cardiopulmonary bypass surgery.6 It has been shown that α, β₁, β₂-adrenergic antagonists fail to improve the balance of oxygen transport, due to ineffective reduction of VO₂ when each selectively used, or attenuation of both VO₂ and DO₂ to a similar degree when combined.3

β₃-adrenoceptors also have both circulatory and metabolic effects, but distinctly different from other adrenergic receptors. In terms of metabolic effect, β₃-adrenergic receptor are richly present in brown adipose tissue. Brown adipose tissue generates heat following stimulation of α-, β₁-, particularly β₃-adrenoceptors by the sympathetic nervous system.7 It plays an essential role in non-shivering thermogenesis, hence VO₂, in newborn humans and some in larger mammals including lambs. The ability to specifically activate or reduce energy expenditure via β₃-adrenoceptor manipulation is of much interest for the thermoregulation in newborns. Using the β₃-adrenergic agonist Zeneca D7114 increased body temperature in moderately hypothermic Caesarean section-delivered lambs.13 Selective and potent β₃-adrenoceptor antagonist SR59230A has been shown to down-regulate uncoupling protein-1 and noradrenaline induced cAMP accumulation, thus reducing thermogenesis in brown adipose tissue in animal experiments including sheep models.13-15 Our findings on the systemic effects of the attenuated rises in central body temperature and VO₂ in newborn lambs treated with SR59230A prior to dopamine infusion are consistent with previous findings at tissue and

Figure 1. The changes of (A) heart rate, (B) cardiac output, (C) systemic oxygen delivery (DO₂), (D) systemic oxygen consumption (VO₂), and (E) oxygen extraction ratio (ERO₂) prior to and following SR59230A infusion with incremental doses of dopamine. Control group received placebo of saline. Notes: P_dose indicates dopamine dose effect during dopamine infusion; and P_dose*group indicates the difference in the rate of change between the two groups.
molecular levels. The improved balance of systemic oxygen transport may, at least partly, contribute the improved myocardial oxygen transport as indicated by the lower mERO2 and higher mERlac in lambs treated with SR59230A be attributed, at least partly, to the improved balance of systemic oxygen transport.

Nonetheless, the specific cardiac effect of SR59230A should also be accounted for. Compared to brown adipose tissue, β3-adrenoreceptors in the heart have been less extensively studied, but have gained increasing interest in recent years. It has been reported that β3-adrenoreceptor stimulation exerts a profound dose-dependent negative inotropic effect in ventricles with decreased myocardial contractility in humans and other species. Hence, it is suggested that β3-adrenoreceptors serves as a “brake” during sympathetic overstimulation to antagonize β1-adrenoreceptors and β2-adrenoreceptors in the heart. In addition, β3-adrenoreceptor stimulation also exerts positive chronotropic effects, probably resulted from reflex mechanisms rather than from a direct stimulation of cardiac β3-adrenoceptors. It might be extrapolated that β3-adrenoreceptor antagonist may increase or maintain the inotropic effect of dopamine, thus myocardial contractility, but at the same time reduce the heart rate. As shown in our data, following SR59230A infusion, there was an immediate reduction in heart rate, but stroke volume was maintained. As a result, CO and DO2 decreased. Nonetheless, during incremental doses of dopamine infusion, the changes in heart rate, CO and stroke volume were not significantly different between the two groups. The lower heart rate and CO, thus reduced myocardial workload, might be attributable to the better balance of myocardial oxygen transport. It is important to note that, despite an initial reduction in heart rate and CO, the greater and continuous reduction in VO2 by SR59230A attenuated the imbalance of systemic oxygen transport at higher doses of dopamine infusion as indicated by the attenuated increase in ERO2.

**Limitations**
There are several limitations in our study. First, stroke volume was used to reflect cardiac contractility. The changes in myocardial contractility in the experiment may be more accurately assessed with direct measurement utilizing techniques such as tissue Doppler by echocardiography or conductance catheter. Secondly, myocardial lactate extraction ratio reflects the overall balance of the complex production and consumption of lactate by myocardium. A high lactate extraction may indicate less lactate production or more lactate consumption, or both. The exact mechanism can only be shown by direct examination of myocardial metabolism. Nonetheless, based on the lower myocardial oxygen extraction ratio in lambs treated with SR59230A, the less lactate production may be likely the case in our study. Thirdly, we did not directly assess the regional changes in oxygen consumption in brown fat tissue. Therefore, the relationship between VO2 and brown fat tissue remains speculative in our study. Forth, this is a pilot study in healthy newborn lambs using only one dose of SR59230A. A dose response study in newborn lambs with myocardial injury and increased VO2, such as newborn subjects following cardiopulmonary bypass may provide more detailed and relevant information about the effects on SR59230A on VO2 and myocardial contractility, thus CO and DO2.

**Conclusion**
Although β3-adrenoceptor antagonist SR59230A tends to induce an initial drop in heart rate and CO, it significantly attenuates the increase in VO2 and hence the imbalance of systemic and myocardial oxygen transport induced by dopamine at higher doses. Further studies are warranted to examine the dose response effect of SR59230A in subjects with myocardial injury and increased VO2 such as those after cardiopulmonary bypass surgery.

**List of Abbreviations**
CO, cardiac output; DO2, systemic O2 delivery; ERO2, oxygen extraction ratio; mERO2, myocardial oxygen extraction ratio; mERlac, myocardial lactate extraction ratio; VO2, systemic O2 consumption.

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The authors declare that we have no competing interests.

**Authors’ Contributions**
RG, conducted the experiment and draft manuscript. PYC, designed the protocol, conducted the
experiment and corrected manuscript. XY, MAA, JN, LGQ, YQL, JM, conducted the experiment, and corrected the manuscript. DBR and IMR, designed the protocol and advised on the animal surgery, and corrected the manuscript. JL, proposed the study, designed the protocol, conducted the experiment, analyzed the data, and corrected the manuscript.

Disclosures

Author(s) have provided signed confirmations to the publisher of their compliance with all applicable legal and ethical obligations in respect to declaration of conflicts of interest, funding, authorship and contributor-ship, and compliance with ethical requirements in respect to treatment of human and animal test subjects. If this article contains identifiable human subject(s) author(s) were required to supply signed patient consent prior to publication. Author(s) have confirmed that the published article is unique and not under consideration nor published by any other publication and that they have consent to reproduce any copyrighted material. The peer reviewers declared no conflicts of interest.

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