FEDERAL UNIVERSITY OF SÃO CARLOS
CENTER OF BIOLOGICAL SCIENCES AND HEALTH
INTERINSTITUTIONAL PROGRAM OF POST-GRADUATION IN PHYSIOLOGICAL SCIENCES UFSCar/UNESP

SAMANTA APARECIDA CASTRO

THE ROLE OF NITRIC OXIDE AND ADRENERGIC STIMULATION ON THE CARDIOVASCULAR ADJUSTMENTS IN SOUTH AMERICAN RATTLESNAKES (*Crotalus durissus*)

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Dissertation presented to the Interinstitutional Postgraduate Program in Physiological Sciences of Federal University of São Carlos, as part of the requirements to obtain the Master's degree in Physiological Sciences.

Advisor: Dr. Cléo Alcantara Costa Leite

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To my parents, Maria and José, for the love, encouragement, dedication, teachings and for offering me the opportunity to study. Thank you for allowing my dream become true!

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I dedicate

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EPIGRAPH

"The Knowledge serves to enchant people,
not to humiliate them."

Mário Sérgio Cortella

"Humility differs from subservience. A humble person knows that it does not know everything. It's the person who knows it is not the only one who knows. It's the one who knows that together with another person they will know a lot more. It's the person who knows that even together, they will never know everything to be known."

Mário Sérgio Cortella

RESUMO

Entender a base da regulação do tônus vascular é importante para compreender a fisiologia cardiovascular de qualquer vertebrado. Investigações recentes em répteis tentaram essa descrição trabalhando em animais anestesiados. Infelizmente, essa abordagem pode ser ineficaz para acessar mecanismos que dependem de algum nível de modulação autonômica. O objetivo deste estudo foi investigar o papel do óxido nítrico (NO) e da estimulação adrenérgica no controle do tônus vascular em répteis. Este estudo utilizou o modelo de cascavel descerebrada desenvolvido para evitar o efeito deletério dos anestésicos na modulação cardiovascular. Descobrimos que o NO é um vasorelaxante eficaz em cascavéis e existe um nível de repouso na produção de NO. O nitroprussiato de sódio (SNP) causou vasodilatação sistêmica, seguida por aumento da condutância sistêmica (G_{sys}) e fluxo sistêmico ($\dot{Q}_{sys}$), e esse resultado foi associado à redução da pressão arterial média sistêmica (PAM_{sys}). Como resposta, o débito cardíaco (DC) foi elevado. Na circulação pulmonar, o SNP aumentou a condutância pulmonar (G_{pul}) e reduziu a pressão arterial média pulmonar (PAM_{pul}), enquanto o fluxo pulmonar ($\dot{Q}_{pul}$) permaneceu inalterado. A vasodilatação sistêmica após a L-arginina (L-Arg) foi semelhante ao efeito da injeção de SNP. Embora não houvesse efeitos da L-Arg na circulação pulmonar. Portanto, em cascavéis, o NO é sintetizado via L-Arg e tem papel parcial na regulação local do tônus vascular sistêmico. Em contraste, a vasculatura pulmonar é menos reativa ao SNP e a produção de NO parece não depender da L-Arg. Adrenalina e fenilefrina causaram vasoconstrição sistêmica que foi abolida pela fentolamina, demonstrando que esta resposta foi mediada por receptores α-adrenérgicos. A injeção de fentolamina causou uma acentuada vasodilatação, seguida de aumento da G_{sys} e $\dot{Q}_{sys}$, e esteve efeito foi associado à redução da PAM_{sys}. A injeção de propranolol promoveu bradicardia, com consequente diminuição do DC e $\dot{Q}_{sys}$, e aumento do tônus vascular sistêmico, sem alteração da G_{sys} e PAM_{sys}. Esses resultados indicam que a modulação adrenérgica, via α-receptores, é quantitativamente mais relevante do que o ramo β-receptor do barorreflexo, para modular a PAM em *Crotalus*. A vasculatura pulmonar é menos responsiva à estimulação adrenérgica. Além disso, a fentolamina foi mais eficaz do que propranolol em alterar a G_{pul}. Portanto, sugerimos que a estimulação adrenérgica, pelo ramo simpático do sistema nervoso autônomo, é mais eficaz do que a atividade cardíaca, em modular o tônus vascular em cascavéis.

Palavras-chave: sistema nervoso autônomo, descerebração, óxido nítrico, répteis, ativação simpática, tônus vascular.

ABSTRACT

Understanding the basis of vascular tone regulation is important to comprehend the cardiovascular physiology of any vertebrate. Recent investigations on reptiles have tried that description working on anesthetized animals. Unfortunatelly, such approach can be ineffective to access mechanisms that rely on some level of autonomic modulation. The aim of this study was to investigate the role of nitric oxide (NO) and of adrenergic stimulation on the control of vascular tone in reptiles. This study used the developed decerebrate rattlesnake model in order to avoid the deleterious effect of anesthetics on cardiovascular modulation. We found that NO is effective vasorelaxing in rattlesnakes and there is a resting level of NO production. Sodium nitroprusside (SNP) caused systemic vasodilation followed by systemic conductance (G_{sys}) and systemic flow ($\dot{Q}_{sys}$) increasing, and it was associated with systemic mean arterial pressure (MAP_{sys}) reduction. As a response, cardiac output (CO) was elevated. In the pulmonary circulation, SNP increased pulmonary conductance (G_{pul}) and reduced pulmonary mean arterial pressure (MAP_{pul}), while pulmonary flow ($\dot{Q}_{pul}$) remained unaffected. The systemic vasodilation after L-arginine (L-Arg) was similar to the effect of SNP injection. Although, there were no effects of L-Arg on the pulmonary circulation. Therefore, in rattlesnakes, NO is synthetized via L-Arg and it has partial role on the local regulation of systemic vascular tone. In contrast, the pulmonary vasculature is less reactive to SNP and also, NO production seems not to be dependent on the L-Arg. Adrenaline and phenylephrine caused systemic vasoconstriction that was abolished by phentolamine, demonstrating this response was mediated by α-adrenergic receptors. Injection of phentolamine caused a marked vasodilatation followed by G_{sys} and $\dot{Q}_{sys}$ increasing, and it was associated with MAP_{sys} reduction. Injection of propranolol promoted bradycardia, with consequent decreasing of CO and $\dot{Q}_{sys}$ and increased of systemic vascular tone, without changing the G_{sys} and MAP_{sys}. These results indicate adrenergic modulation via α-receptors is quantitativelly more relevant to modulate MAP than the β-receptors branch of barorreflex in *Crotalus*. Pulmonary vasculature is less responsive to adrenergic stimulation. Also, phentolamine was more effective in alter G_{pul} than propranolol. Therefore, we suggest that the adrenergic stimulation by the sympathetic branch of the autonomic nervous system is more effective in modulate vascular tone than cardiac activity in rattlesnakes.

Key words: autonomic nervous system, decerebration, nitric oxide, reptiles, sympathetic activation, vascular tone.

1. GENERAL INTRODUCTION

The cardiovascular system of vertebrates is modulated by the autonomic nervous system (ANS). Thus, heart and vessels appear to be under ANS action in all groups analyzed (Taylor et al., 1999; 2014). In reptiles, the heart and vessels receive direct innervation of excitatory cholinergic parasympathetic and adrenergic inhibitory fibers (Morris and Nilsson, 1994). The vascular system of reptiles can receive excitatory innervation of sympathetic fibers in both central and peripheral vasculature, moreover, the parasympathetic excitatory innervation on the central vasculature, especially on the pulmonary circuit (Taylor et al., 1999; 2009; Leite et al., 2013; 2014). Although this general description is available, there is little information about mechanisms of cardiovascular adjustments in reptiles. Information from this group is of fundamental importance to base any theory on the evolution of mechanisms and processes on the vertebrate group. Thus, the study of vascular tone regulation of the systemic and pulmonary circuits has important implications for understanding the physiology of reptiles and allows a better understanding of the functioning of mechanisms for cardiovascular adjustments in vertebrates.

The cardiac anatomy of reptiles is especially interesting because its ventricle is only partially divided, allowing selective distribution of blood between the systemic and pulmonary circulations (intracardiac shunting) (Hicks, 1998). The possibility of mixing rich and oxygen-poor blood flows has long been viewed as an inefficient cardiovascular mechanism, since it was different from that found in birds and mammals (Foxon, 1955). However, this thinking changed when studies showed that degree of blood mixing within the reptilian heart is controlled by the ANS, and that it varies with the physiological state of the animal in response to alterations on the internal or external environment (Hicks and Malvin 1992; Comeau and Hicks 1994; Hicks and Comeau 1994; Hicks 1994).

Currently, it is well established that cholinergic vagal control of the pulmonary artery determines variations in intracardiac shunting patterns by affecting pulmonary circuit conductance (Johansen and Burggren, 1980; Hicks, 1998; Taylor et al, 2009). In addition, there is modulation of vascular conductance through adrenergic stimulation on the systemic circulation, however, it is not known whether this stimulation contributed to the control of intracardiac shunting or how much it may affect pulmonary conductance in different reptile species (Lillywhite and Donald, 1994). Similarly, several studies have focused on characterizing the role of nitric oxide (NO) on the cardiovascular system of reptiles (Crossley et al. 2000; Axelsson et al. 2001; Galli et al. 2005b; Donald and Broughton 2005; Skovgaard et al., 2005b; Broughton and Donald, 2007). In some of these studies, it was reported that inhibition of NO synthesis increased systemic vascular tone, demonstrating that NO release contributes to the basal regulation of systemic conductance (Knight and Burnstock, 1993; Axelsson et al. 2001). However, these data show only a qualitative analysis of the presence of this mechanism.

Adrenergic and NO influences have been observed experimentally in anesthetized rattlesnakes (Galli et al., 2005b; Galli et al., 2007). The use of anesthesia to allow more invasive instrumentations, allowing multiple concomitant measurements, is relevant from a qualitative point of view. However, quantitative inferences, limits of adjustments on the investigated systems, or any integration in response to challenges provided by changes in internal and external environments is very limited, since ANS is dysfunctional due to the anesthetic used. In this context, the use of rattlesnake as a study model is due the amount of information available about the cardiovascular system of this specie and by the representativeness that it presents in the order Squamata. Thus, the study model with the use of decerebrate rattlesnakes can have important implications for research on the cardiovascular physiology of reptiles. Since, it allows investigations of the animal's autonomic function, with instrumentation for multiple simultaneous measurements, without the use of anesthesia.

Therefore, we worked with a series of hypotheses in this investigation: (1) the animal model, using the decerebrate rattlesnake is an efficient method for obtaining data related to the regulatory processes of the ANS, since in such model it is operative. (2) we also suggest that the action of NO on vascular adjustments is marked and relevant on both, systemic and pulmonary circuits. (3) in addition, we believe that adrenergic stimulation has an effect on both circuits and is capable of affecting intracardiac shunting by providing a greater relative reduction in systemic conductance compared to pulmonary conductance. (4) we also believe that relevance of vascular autonomic control is underestimated due to the use of anesthetized animal models and, therefore, unprovided of autonomic tone and functional support for presenting regulatory adjustments. This research has relevance for inserting a new form to analyze the presence and effectiveness of cardiovascular adjustments and it questions some of the data previously produced with anesthetized animals.

2. CONCLUSIONS

We suggest that in *Crotalus durissus*, NO is synthesized via the L-arginine (L-Arg) mechanism and has a partial role in the local regulation of vascular tone on the systemic circulation. In contrast, the pulmonary vasculature is less reactive to sodium nitroprusside (SNP) and it is unresponsive to L-Arg, demonstrating that, in this circuit, NO production seems not to be dependent on the L-Arg. Thus, we suggest that NO-mediated vasodilation there is an important role on systemic cardiovascular adjustments on the resting condition in Squamata. And, the vascular adjustments of the pulmonary circuit promoted by NO could be relevant in other physiological conditions, with higher energy demand.

Adrenergic stimulation, on the other hand, there is a fundamental role in the local regulation of systemic vascular tone. While, pulmonary vasculature is less responsive to adrenergic agonists. In addition, we can indicate that α-adrenergic receptors are more efficient than β-adrenergic receptors in controlling blood pressure in *Crotalus*. The lack of effects of adrenergic stimulation on cardiac parameters also indicates that adrenergic agonists have more vascular action than cardiac action in controlling systemic and pulmonary vascular tone in this specie. The lack of change in pulmonary conductance and Net-Shunt demonstrates that parasympathetic regulation of the cardiovascular system is more effective than sympathetic regulation for controlling intracardiac shunting in *Crotalus*. The data presented so far allow safe discussions about the importance of ANS for the control of vascular tone in reptiles and demonstrates that the model of the decerebrate rattlesnake has enabled to obtain consistent cardiovascular data. Thus, decerebration proved to be a great alternative for studies involving autonomic mechanisms, emphasizing its importance and possible implications for area of evolutionary physiology.

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