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ANDRÉ SALES BARRETO

MEDIAÇÃO NEURAL NA HIPOTENSÃO PÓS-EXERCÍCIO EM RATOS HIPERTENSOS

ARACAJU – SE

2014

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Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde do Núcleo de Pós-Graduação em Medicina da Universidade Federal de Sergipe para obtenção do título de Doutor em Ciências da Saúde. Área de concentração: Neurociências.

Orientador: Prof. Dr. Márcio Roberto Viana Santos

Co-orientador: Prof. Dr. Valter Joviniano Santana Filho

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PARECER

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Dedico o fruto deste trabalho a minha amada esposa, companheira e amiga Rosana, aos meus amados filhos Giovana e Samuel e aos meus pais Eliel e Valdecí sinônimos de amor incondicional. Vocês são minha fonte de inspiração e alegria.

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Mediação neural na hipotensão pós-exercício em ratos hipertensos. BARRETO, André Sales. Universidade Federal de Sergipe, Aracaju, 2014, p. 120.

RESUMO

A redução sustentada da pressão arterial após uma única sessão de exercício aeróbico ou resistido (ER) tem ganhado significativa relevância clínica em indivíduos hipertensos. Esse fenômeno é conhecido como hipotensão pós-exercício (HPE). No entanto, os mecanismos neurais que levam a HPE ainda necessitam serem melhor compreendidos, especialmente decorrentes do ER. Neste sentido, o presente trabalho buscou revisar os mecanismos neurais envolvidos na HPE e avaliar as alterações hemodinâmicas e controle autonômico provocadas pelo ER em ratos hipertensos induzidos por Nω-Nitro-L-arginina metil éster (L-NAME). A tese é composta por dois capítulos, constituídos de uma revisão sistemática e um artigo original. Inicialmente foi elaborada a revisão sistemática *"A systematic review of neural mechanisms involved on post-exercise hypotension in hypertensive animals"*, com busca dos artigos nos bancos de dados LILACS, PUBMED e EMBASE, a qual descreve uma visão geral dos mecanismos neurais envolvidos na HPE em estudos realizados com animais hipertensos. Esses estudos demonstraram que a presença de aferência cardiovascular, estímulo da aferência muscular esquelética durante o exercício e modulações suprabulbares são fundamentais para a expressão da HPE. Após a realização dos protocolos experimentais foi elaborado o artigo "*Arterial Baroreflex participates in the post-resistance exercise hypotension in L-NAME-induced hypertensive rats*". Este artigo demonstrou que o aumento da sensibilidade do barorreflexo arterial induzido pelo ER desempenha um papel crucial na HPE seguida de bradicardia, provavelmente através da inibição simpática cardíaca e vascular. Juntos, esses achados permitem concluir que a participação de mecanismos neurais são importantes para a manifestação da HPE induzido por ambos os tipos de exercício aeróbico e resistido em ratos hipertensos.

Palavras-chave: hipertensão; hipotensão pós-exercício; exercício; condicionamento físico animal; sistema nervoso autônomo.

Neural mediation in post-exercise hypotension in hypertensive rats. BARRETO, André Sales. Universidade Federal de Sergipe, Aracaju, 2014, p. 120.

ABSTRACT

The sustained reduction in blood pressure after a single bout of aerobic or resistance exercise (RE) has gained significant clinical relevance in hypertensive individuals. This phenomenon is known as post-exercise hypotension (PEH). However, the neural mechanisms that lead to HPE still need to be better understood, particularly arising from the ER. In this sense, the present study sought to review the neural mechanisms involved in HPE and evaluate the hemodynamic and autonomic control changes induced by ER in hypertensive rats induced by Nω-nitro-L-arginine methyl ester (L-NAME). The thesis consists of two chapters, which are systematic review and an original article. Initially was elaborated a systematic review "A systematic review of neural mechanisms involved on post-exercise hypotension in hypertensive animals", with search for articles in LILACS, EMBASE and PUBMED database, which describes an overview of the neural mechanisms involved in HPE in studies of hypertensive animals. These studies demonstrated the presence of cardiovascular afferents, afferent skeletal muscle stimulation during exercise and bulbar or suprabulbar modulations are fundamental to the expression of HPE. After completion of the experimental protocols the article: "Arterial Baroreflex participates in the post-resistance exercise hypotension in L-NAME-induced hypertensive rats", was presented. This paper demonstrated that the increased baroreflex arterial sensitibity RE-induced plays a crucial role in PEH followed by bradycardia, probably through cardiac and vascular sympathetic inhibition. Together, these findings show that the involvement of neural mechanisms are important for the manifestation of PEH induced by both aerobic and resistance exercise in hypertensive rats.

Keywords: hypertension; post-exercise hypotension; exercise; physical conditioning, animal; autonomic nervous system.

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Arterial Baroreflex participates in the post-resistance exercise hypotension in L-NAME-induced hypertensive rats:

LISTA DE ABREVIATURAS

AH	Arterial hypertension
ANS	Autonomic nervous system
AP	Arterial pressure
AVP	Arginine vasopressina
BRS	Barorreflex sensitivity
BP	Blood pressure
CAB	Cardiac autonomic balance
CVLM	Caudal ventrolateral medula
CO	Cardiac output
DA	Dopamine
DAP	Diastolic arterial pressure
D2-R	Dopamine 2 receptor
DBS	Dorsal brainstem
DMV	Dorsal motor nucleus of the vagus
EA	Exercício aeróbico
EN	Exercised normotensive animals
EH	Exercised hypertensive animals
ER	Exercício resistido
FC	Frequência cardíaca
GABA	Gamma-Aminobutyric acid
HAS	Hipertensão arterial sistêmia
HPE	Hipotensão pós-exercício
HF	High frequency component from pulse interval
HR	Heart rate
LF	Low frenquency component from pulse interval
LF/HF ratio	Low frequency/high frequency ratio
LFsys	Low frequency component from systolic arterial pressure
L-NAME	Nω-Nitro-L-arginine methyl ester
LSNA	Lumbar sympathetic neural activity
MAP	Mean arterial pressure
NA	Nucleus ambiguous

NK-1 R	Neurokinin 1 receptor
NTS	Nucleus tractus Solitarii
NO	Nitric oxide
OT	Oxytocin
OT mRNA	Oxytocin messenger Ribonucleic acid
OT-R	Oxytocin receptor
PA	Pressão arterial
PEH	Post-exercise hypotension
PI	Pulse interval
PVN	Núcleo paraventricular do hipotálamo
PVR	Peripheral vascular resistance
RE	Resistance exercise
RM	Repetition maximum
RVLM	Rostral ventrolateral medula
RVP	Resistência vascular periférica
SAP	Systolic arterial pressure
SBP-LFamp	Systolic blood pressure – low frequency power amplitude
SBR	Sensibilidade do barorreflexo
SEM	Standard error mean
SHR	Spontaneously hypertensive rat
SH	Sham hypertensive animals
SN	Sham normotensive animals
SON	Supraoptic nucleus
TPR	Total peripheral resistance
V1-R	Vasopressin-1 receptor
VLF	Very low frequency component from pulse interval

SUMÁRIO

INTRODUÇÃO

1 INTRODUÇÃO

A hipertensão arterial sistêmica (HAS) é geralmente definida pela presença de uma elevação crônica da pressão arterial (PA) acima de um determinado valor limite (GILES et al., 2005, 2009; MANCIA et al., 2013). HAS é fortemente associada com anormalidades funcionais e estruturais cardiovasculares que danificam coração, vasculatura, rins, cérebro e outros órgãos, os quais conduzem a morbidade e morte prematura (GILES et al., 2005, 2009; MANCIA et al., 2013). Complicações da HAS são responsáveis por 9,4 milhões de mortes no mundo a cada ano (LIM et al., 2012). Em 2008, estimou-se no mundo que aproximadamente 40% dos adultos com idades acima de 25 anos tinham sido diagnosticados com HAS, e o número de pessoas com a doença passou de 600 milhões em 1980, para 1 bilhão em 2008 (WHO, 2010).

No Brasil, estima-se que na população urbana adulta, a prevalência da HAS é de 17 milhões de portadores e aproximadamente 6 milhões possuem idade igual ou superior à 40 anos. Em Sergipe, calcula-se que existam aproximadamente 162,5 mil hipertensos, sendo que 30% deles não são acompanhados pelos Programas de Saúde da Família nem constam no Sistema de Informação da Atenção Básica (MINISTÉRIO DA SAÚDE, 2010). Com estas estimativas e devido ao seu considerável custo econômico, o tratamento da HAS é um grande desafio para a saúde pública especialmente dos países em desenvolvimento (MITTAL; SINGH, 2010).

Muitas evidências indicam que a ativação central do sistema nervoso simpático, tem um papel importante na patogênese ou manutenção da HAS (COFFMAN, 2011; ESLER, 2010, 2011; GRASSI; SERAVALLE; QUARTI-TREVANO, 2010; GRASSI, 2009, 2010; MALPAS, 2010). Estes resultados têm atraído o interesse de muitos pesquisadores nos mecanismos cerebrais que levam a um maior fluxo simpático central (CHAN; CHAN, 2012; FISHER; FADEL, 2010; GABOR; LEENEN, 2012a; GUYENET, 2006; HIROOKA et al., 2011).

Ajustes imediatos da PA são controlados e reforçados pelo sistema nervoso autônomo. Aferências periféricas como barorreceptores e quimiorreceptores arteriais, além de receptores cardiopulmonares sinalizam para uma área bulbar específica do sistema nervoso central responsável pelo controle da PA conhecida como núcleo do trato solitário (NTS), o primeiro local de integração da informação periférica (DAMPNEY, 1994; ZANUTTO; VALENTINUZZI; SEGURA, 2010).

O NTS apresenta diversas projeções excitatórias bulbares para grupos de neurônios pré-ganglionares parassimpáticos como motor dorsal do vago (DMV) e ambíguo (NA) que inervam o coração e também para neurônios do bulbo ventrolateral caudal (CVLM), os quais

inibem neurônios do bulbo ventrolateral rostral (RVLM). O RVLM é a principal origem do tônus simpático cardiovascular, seus neurônio pré-motores projetam-se aos neurônios pré-ganglionares na coluna intermédio-lateral da medula espinhal e destes aos pós-ganglionares que inervam coração e vasos (DAMPNEY, 1994; ZANUTTO; VALENTINUZZI; SEGURA, 2010). Essa alça reflexa bulbar em normotensos corrige, instantaneamente, os desvios da PA, no entanto em hipertensos tanto a sensibilidade da aferência quanto a integração dos diferentes núcleos cardiovasculares centrais estão alterados reforçando a predominância da atividade simpática sobre a parassimpática (GABOR; LEENEN, 2012b; SVED; ITO; SVED, 2003; THOMAS et al., 2013).

Além disso, projeções suprabulbares recíprocas do NTS para regiões específicas do hipotálamo como núcleo paraventricular (PVN) também participam da modulação da PA (DAMPNEY, 1994; PALKOVITS, 1999). Neurônios pré-autonômicos parvocelulares do PVN enviam respostas através de projeções descendentes vasopressinérgicas e ocitocinérgicas para o NTS, DMV, NA e RVLM de forma a integrar e modular ajustes finos do controle bulbar (LANDGRAF et al., 1990; SAWCHENKO; SWANSON, 1982), especialmente em condições desafiadoras como o exercício físico e hipertensão arterial (MARTINS et al., 2005; MICHELINI, 2007a, 2007b). Essa alça reflexa é conhecida como suprabulbar ou secundária e sua disfunção também ratifica a condição hipertensiva (GABOR; LEENEN, 2012b).

Apesar dos esforços, após décadas de tentativas no controle da HAS com uso de abordagens farmacológicas, apenas 31% dos indivíduos adultos são adequadamente controlados nos Estados Unidos da América (LLOYD-JONES et al., 2009). Essa falta de avanço significativo no manejo farmacológico tem levado a ampliação de abordagens não-farmacológicas para melhorar o gerenciamento ou reduzir a prevalência da hipertensão (APPEL, 1999).

Neste contexto, mudanças de estilo de vida adequadas são fundamentais para a prevenção e tratamento da HAS. Estudos clínicos mostram que os efeitos anti-hipertensivos de modificações do estilo de vida específicas podem ser equivalentes a monoterapia por medicamento, embora não deva-se excluir o tratamento farmacológico (ELMER et al., 2006). Uma das mudanças de estilo de vida recomendada é a prática regular de exercício físico (DICKINSON et al., 2006). As recomendações atuais à prescrição de exercício para esse grupo populacional é a prática de exercício aeróbico (EA) complementado pelo exercício resistido (ER) ambos com intensidade moderada (PESCATELLO et al., 2004).

O exercício físico crônico é associado a uma variedade de ajustes cardiovasculares funcionais e estruturais benéficos aos indivíduos hipertensos que resultam na redução da PA, frequência cardíaca (FC) de repouso (CORNELISSEN; FAGARD, 2005; CORNELISSEN; SMART, 2013; CORNELISSEN et al., 2011; KELLEY; KELLEY, 2010; PESCATELLO et al., 2004) e mortalidade por complicação cardiovascular (ENGSTRÖM; HEDBLAD; JANZON, 1999; TAYLOR et al., 2006; WISLØFF et al., 2006). Recentemente também foi demonstrado que o ER crônico de moderada intensidade (50% de 1 RM), controlou a PA e reduziu a sensibilidade α_1-adrenérgica em artéria mesentérica sem endotélio de ratos hipertensos induzidos cronicamente pela Nω-Nitro-L-arginina metil éster (L-NAME) (ARAUJO et al., 2013).

No entanto, recentemente tem sido dada atenção não apenas aos efeitos crônicos, mas também aos efeitos agudos do exercício (LIZARDO et al., 2008). Imediatamente após um única sessão de exercício, os níveis de PA diminuem em poucos minutos e persistem por horas em relação aos níveis pré-exercício (BRANDÃO RONDON et al., 2002; HALLIWILL, 2001; KENNEY; SEALS, 1993; MACDONALD, 2002; MELO et al., 2006; MOTA et al., 2009; QUEIROZ et al., 2009, 2013; REZK et al., 2006). Este fenômeno é conhecido como hipotensão pós-exercício (HPE) e tem-se mostrado de grande relevância clínica para o tratamento e prevenção da HAS (HALLIWILL, 2001; KENNEY; SEALS, 1993; MACDONALD, 2002; PESCATELLO et al., 2004).

A HPE tem sido associada a uma redução sustentada da resistência vascular periférica (RVP) e um aumento na condutância vascular sistêmica (HAGBERG; MONTAIN; MARTIN, 1987; HALLIWILL; TAYLOR; ECKBERG, 1996; KULICS; COLLINS; DICARLO, 1999). Tais respostas foram atribuídas a mecanismos locais como redução na responssividade dos receptores α-adrenérgicos vasculares após exercício aeróbico (RAO; COLLINS; DICARLO, 2002), aumento de relaxamento vascular dependente do endotélio em animais hipertensos e normotensos após exercício resistido (FARIA et al., 2010; FONTES et al., 2014; LIZARDO et al., 2008). Além disso, mecanismos neurais como aumento da aferência cardiopulmonar, sensibilidade do barorreflexo (COLLINS; DICARLO, 1993; MINAMI et al., 2006; MOTA et al., 2013; SILVA et al., 1997) e redução da atividade simpática cardiovascular também são associados a HPE (FLORAS et al., 1989; HALLIWILL; TAYLOR; ECKBERG, 1996; KAJEKAR et al., 2002; KULICS; COLLINS; DICARLO, 1999).

Dentre os fatores neurais que mediam a HPE o barorreflexo arterial tem se mostrado essencial. Em animais que foram submetidos a desnervação sino aórtica, ou seja, que retiraram

a aferência do barorreflexo arterial, houve total bloqueio da HPE e bradicardia reflexa (CHANDLER; DICARLO, 1997; CHANDLER; RODENBAUGH; DICARLO, 1998). Esses estudos demonstram que é necessário haver um barorreflexo funcional para que haja HPE.

No entanto, a complexa interação dos neurônios barorreceptores com centros cardiovasculares cerebrais tem-se permitido especular que tanto a integração de aferências musculares, cardiopulmonares e de quimiorreceptores no núcleo do trato solitário (NTS) (CHEN et al., 2002, 2009), quanto modulação suprabulbar (AKIYAMA; SUTOO, 1999; COLLINS; RODENBAUGH; DICARLO, 2001), especialmente no núcleo paraventricular (PVN) do hipotálamo, podem influenciar a resposta barorreflexa arterial, e portanto, na manifestação da HPE em hipertensos.

Embora o ER seja uma das recomendações no tratamento da hipertensão e induza significativa HPE em diversos trabalhos com humanos (BRITO et al., 2011; MELO et al., 2006; MORAES et al., 2012; MOTA et al., 2013; WILLIAMS et al., 2007), estudos em animais, os quais poderiam esclarecer melhor os mecanismos envolvidos neste fenômeno, têm sido pouco explorados (FARIA et al., 2010; LIZARDO et al., 2008). O recrutamento da aferência muscular esquelética e a necessidade de adaptações hemodinâmicas imediatas requeridas durante o exercício físico sugerem que importantes vias neurais possam estar envolvidas no completo desenvolvimento da HPE durante o período de recuperação pós-exercício em animais hipertensos (MINAMI et al., 2006).

Para avaliar possível participação neural na HPE, modelos experimentais de hipertensão têm sido utilizados. O modelo experimental de hipertensão mais utilizado para investigar tal fenômeno são os ratos espontaneamente hipertensos (SHR) devido as suas similaridades quanto a hipertensão essencial, além da magnitude e duração da HPE encontradas em humanos (MELO et al., 2006; TRIPPODO; FROHLICH, 1981). O aumento da MAP e FC encontrado neste modelo é principalmente produzido pela hiperatividade simpática (DICKHOUT; LEE, 1998; TÖRÖK, 2008). Um outro modelo de hipertensão animal induzido pela administração crônica de L-NAME, um inibidor da síntase do óxido nítrico (NOS), possui como principal característica a deficiência da formação de oxido nítrico (NO) no endotélio vascular, embora outros mecanismos possam estar envolvidos na manutenção da PA elevada como o aumento da descarga simpática (TÖRÖK, 2008).

Diversos estudos tem atribuído à HPE principalmente a mecanismos vasodilatadores locais (HALLIWILL et al., 2013; LIZARDO et al., 2008). Nesse sentido, a utilização de um modelo de hipertensão que iniba predominantemente a expressão de uma importante via

periférica vasodilatadora, como a deficiência na biodisponibilidade de NO pelo L-NAME, poderia contribuir para a melhor compreensão de mecanismos neurais centrais envolvidos neste efeito.

Não obstante, o entendimento dos mecanismos neurais subjacentes à HPE induzidas pelo ER pode ser um importante passo na elaboração de estratégias, quanto à sua prescrição, em indivíduos hipertensos. Além disso, o ER tem apresentado significativa segurança cardiovascular avaliada através de medida indireta do trabalho miocárdico, assegurando seu uso precoce em diferentes situações patológicas, inclusive quando comparado ao EA de esforço semelhante (FARINATTI; ASSIS, 2012; POLLOCK et al., 2000). A tradicional percepção de que o ER é prejudicial para pacientes cardíacos não é suportado por dados científicos (ADAMS et al., 2006). Desta forma, é possível ratificar a prescrição do exercício permitindo uma maior ênfase no ER nesse grupo populacional.

Por conseguinte, o propósito deste estudo foi verificar a participação de mecanismos neurais na hipotensão pós-exercício em ratos hipertensos.

OBJETIVOS

2 OBJETIVOS

2.1 OBJETIVO GERAL

- Analisar mecanismos neurais envolvidos na hipotensão pós-exercício em ratos hipertensos.

2.2 OBJETIVOS ESPECÍFICOS

- Realizar um levantamento bibliográfico, através da elaboração de uma revisão sistemática, acerca dos mecanismos neurais envolvidos na HPE em animais hipertensos;
- Avaliar as alterações na PA e FC após uma única sessão de ER de intensidade moderada em ratos hipertensos induzidos por L-NAME;
- Avaliar as alterações na sensibilidade do barorreflexo e modulação autonômica cardiovascular após uma única sessão de ER de intensidade moderada em ratos hipertensos induzidos por L-NAME.

RESULTADOS

3 RESULTADOS

3.1 A SYSTEMATIC REVIEW ABOUT NEURAL MECHANISMS INVOLVED ON POST -EXERCISE HYPOTENSION IN HYPERTENSIVE ANIMALS

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A systematic review about neural mechanisms involved on post-exercise hypotension in hypertensive animals

Running Title: Neural mechanisms on post-exercise hypotension

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Abreviation list

AH	Arterial hypertension
AVP	Arginine vasopressina
BP	Blood pressure
CO	Cardiac output
D2-R	Dopamine 2 receptor
DBS	Dorsal brainstem
DMV	Dorsal motor nucleus of the vagus
GABA	Gamma-Aminobutyric acid
HR	Heart rate
L-NAME	Nω-nitro-L-arginine methyl ester
MAP	Mean arterial pressure
mRNA	Menssenger ribonucleic acid
NA	Nucleus ambiguus
NK-1 R	Neurokinin-1 receptor
NO	Nitric oxide
NTS	Nucleus tractu solitarii
OT	Oxitocin
PEH	Post-exercise hypotension
PVN	Paraventricular nucleus of hypothalamus
RVLM	Rostral ventrolateral medulla
SHR	Spontaneously hypertensive rat
SON	Supraoptic nucleus
TPR	Total peripheral resistance
V1-R	Vasopressin-1 receptor

Abstract

Recently attention has been given to the effects of a single bout of acute exercise on blood pressure (BP) reduction. This phenomenon, known as post-exercise hypotension (PEH), can be considered an important strategy to help control BP at rest, especially in hypertensive individuals. The analysis of neural mechanisms involved in PEH suggested by animal studies could contribute to a better understanding of this phenomenon in hypertensive humans. Thus our systematic review was performed to provides an overview of the neural mechanisms involved in PEH in hypertensive animal studies. In this search, the terms “hypertension”; “post-exercise hypotension”; “exercise”; “physical conditioning, animal”; “weight lifting”; “resistance training”; “autonomic nervous system”; “autonomic nervous system diseases”; “central nervous system”; “hypothalamus”; “solitary nucleus”; “medulla oblongata” were used to retrieve published articles in LILACS, PUBMED and EMBASE until Jan, 2014. Fifteen papers were found concerning neural mechanisms involved on PEH in hypertensive animals. This review showed evidence of several neural mechanisms involved in PEH in hypertensive rats. The data reviewed here suggest that the complexity of neural network in the expression of PEH in hypertensive rats involves different neural mechanisms. Nevertheless, the presence of functional baroreflex, skeletal muscle afferents activation during exercise and bulbar or suprabulbar modulations have received great attention.

Keywords: hypertension; post-exercise hypotension; exercise; physical conditioning, animal; autonomic nervous system; central nervous system.

INTRODUCTION

Arterial hypertension (AH) is usually defined by the presence of a chronic elevation of systemic arterial pressure above a certain threshold value. Its progression is strongly associated with functional and structural cardiac and vascular abnormalities that damage the heart, kidneys, brain, vasculature, and other organs leading to premature morbidity and death.[1–3] After decades of advances in pharmacological treatment, only 31% of hypertensive adults are adequately controlled in the United States.[4] This low development in the pharmacological approaches has motivated the search for non-pharmacological alternatives for the treatment and prevention of AH.[5]

Appropriate lifestyle changes can be the cornerstone for prevention and treatment of AH. Clinical studies have shown that lowering blood pressure (BP) due to changes in lifestyle are equivalent to pharmacological monotherapy[6] although should not exclude it. One of the main recommended lifestyle measures is regular physical exercise practice.[7] Exercise training has been associated with a variety of beneficial cardiovascular adjustments in hypertensive individuals as the significant reduction in BP and heart rate (HR) levels.[8–11]

On the other hand, recent attention has been given not only to chronic effects from exercise, but also to the acute effects from a single bout of exercise. After a single bout of exercise, BP levels decrease within minutes and persist for several hours when compared to pre-exercise values.[12,13] This phenomenon is called post-exercise hypotension (PEH) and can be considered an important strategy to help control resting BP, especially in hypertensive individuals.[13,14]

During the exercise recovery period, neural mechanisms contribute to the fall in BP exercise-induced. [15] In this context, the analysis of neural mechanisms involved in PEH suggested by animal studies could contribute to a better understanding of this phenomenon in

hypertensive humans. Therefore, the aim of this study was to conduct a systematic review of the literature about the neural mechanisms involved on PEH in hypertensive animals.

METHODS

The present systematic review was conducted according to the guidelines for Transparent Reporting of Systematic Reviews and Meta-Analyses (PRISMA statement).[16]

Search Strategy

Three databases (internet sources) were used to search for appropriate papers that fulfilled the study purpose. Those included the National Library of Medicine, Excerpta Medical Database by Elsevier (EMBASE), and Latin American and Caribbean Health Sciences (LILACS), using different combinations of the following keywords considering MeSH and DeCS terms: hypertension; post-exercise hypotension; exercise; physical conditioning, animal; weight lifting; resistance training; autonomic nervous system; autonomic nervous system diseases; central nervous system; hypothalamus; nucleus tractus solitarii; medulla oblongata.

The databases were searched for studies conducted in the period up to and including Jan, 2014. The structured search strategy was designed to include any published paper that evaluated neural mechanisms involved on PEH in hypertensive. Citations were manually limited to animal studies. Additional papers were included in our study after analyses of all references from the selected articles. We did not contact investigators, nor did we attempt to identify unpublished data.

Study Selection

All electronic search titles, selected abstracts, and full-text articles were independently reviewed at least by for two reviewers (A.S.B., R.S.S.B. and J.S.S.Q.). Disagreements on study

inclusion/exclusion were resolved with the reach of a consensus. The following inclusion criteria were applied: acute effect post-exercise, only hypertensive subjects, and neural mechanisms of BP control. Studies were excluded according to the following exclusion criteria: studies in humans, studies in hypertensive disease not isolated, review articles, meta-analyses, abstracts, conference proceedings, editorials/letters, case reports and monograph (Fig. 1).

INSERT FIGURE 1

Data Extraction

Data were extracted by one reviewer using standardized forms and were checked by a second reviewer. Extracted information included data regarding the hypothesis, animal, hypertension model, exercise, exercise protocol, time of monitoring after exercise, valued parameter settings, results post-exercise and neural mechanisms.

RESULTS

A total of 1,241 abstracts/citations were identified from electronic and manual searches for preliminary review. The primary search identified 1,240 articles, with 1,113 from PUBMED, 119 from EMBASE, 8 from LILACS and one from manual search. After removal of duplicates and screening for relevant titles and abstracts, a total of 42 articles were submitted for a full-text review. Fifteen articles met the inclusion and exclusion criteria established. A flow chart illustrating the progress of study selection and article number at each stage is shown (Fig. 1).

Of the 15 studies finally selected (Table 1), it was observed that most research has been done for over a decade (80%) and only two of these in the last 5 years (13%). Regarding the

hypotheses that motivated researchers in conducting such studies, were considered both afferent (33%) and efferent pathways (27%) and probable central modulations (40%). Furthermore, most of the studies analyzed the participation up to medulla oblongata (87%), while only few studies evaluated the suprabulbar pathways (13%).

INSERT TABLE 1

Young adult male animals (~12 weeks-old) were used in most studies (93%) and only three studies were conducted also with female animals. All studies used spontaneously hypertensive rats (SHR) as hypertension model.

Concerning the exercise, all protocols were conducted in sedentary animals, which it were predominantly from aerobic exercise (93%), mainly using motor-driven treadmill. Only one study used resistance exercise (RE) through a squat apparatus. In most studies the intensity of aerobic exercise was evaluated by velocity (10-15 m/min) and inclination (10^{o}) of treadmill and classified as mild to moderate. Exercise volumes were determinated by duration of aerobic exercise performed, which were betweeen 30-60 min. Volume of RE protocol was determinated by sets (10) and repetitions (10) numbers. Hemodynamic monitoring time after exercise in most studies (63%) lasted at least 60 min.

Mean arterial pressure (MAP) and HR were the most measured hemodynamic parameters. Only one study also assessed others parameters such as cardiac output (CO) and total peripheral resistance (TPR). Regarding nervous system parameters, were evaluated both afferents, central integration and efferents pathways. Studies which evaluated the afferent inputs the most analyzed was the arterial baroreflex (80%). Nucleus tractus solitarii (NTS) (50%) and rostral ventral lateral medulla (RVLM) (33%) were the central regions of integration

most assessed. About efferent responses, the cardiac sympathetic and parasympathetic tonus mensured were the most studied (75%) compared to vascular neural control evaluations.

Regarding hemodynamic results all studies showed a significant reduction in MAP after exercise about 20-30 mmHg compared to pre-exercise period, even after RE protocol. The fall in BP after exercise was observed from 10 to 20 minutes in most studies (75%). Several studies (46%) showed significant bradycardia after a single bout of mild to moderate aerobic exercise, which began at least 10 minutes post-exercise. Moreover, both these falls in MAP and HR persisted throughout the monitoring time.

The neural mechanisms involved in post-exercise hypotension according to evaluated studies, were mediated through the: increased arterial baroreflex sensitivity, or its reseting of operating point; augmented cardiac and vascular sympathoinhibition; increased enkephalin synthesis in NTS and RVLM; enhanced dopamine synthesis in the brain and dopamine D_2 receptor activation; increased central vasopressin receptor (V_1-R) activation; augmented GABA signalization in RVLM neurons; increased substance P and neurokinin-1 receptor (NK1-R) activation in the NTS during exercise in addition to NK1-R internalization in GABA interneurons from NTS.

DISCUSSION

This review found evidence that neural mechanisms play a important role in the development of PEH induced by a single bout of aerobic exercise in hypertensive rats. PEH is well demonstrated in both hypertensive humans[17–20] and animals.[21–25] Several pathways have been suggested and particular attention has been given to the central integration of cardiovascular control nuclei such as the NTS and RVLM.[23,26,27] In addition, potential descending pathways from the hypothalamus has been demonstrated in hypertensive animals.[28,29]

Surprisingly, in this review only three of 15 final selected studies were conducted over the last decade. BP is a variable influenced by many local and neurohumoral factors and the effective contribution of each mechanism in PEH is not yet fully elucidated. Undoubtedly, due to the complexity of integration of neural network in the modulation of BP more studies are needed to better understand this phenomenon. On the other hand, attribution of greater role to local vasodilator mechanisms in development and duration of HPE by some studies[25,30,31] may have contributed to the reduction of most recent studies involving neural mechanisms.

The animal model of hypertension used in all studies was the SHR, a experimental model which resembles to human essential hypertension.[32–34] This hypertension model shows increases in both MAP and HR produced mainly by autonomic dysfunction, which is characterized by sympathetic overactivity and cardiac vagal reflex attenuation..[35] Others studies have demonstrated the involvement of changes in neural networks as medulla oblongata [36] and hypothalamus[36,37], reinforcing the importance of neural modulation alteration of BP in this model. However, this experimental model does not present a major local vascular component in hypertension pathogenesis.[38,39] The use of other experimental models, which have great local vascular component in the onset of hypertension as induced by Nω-nitro-L-arginine methyl ester (L-NAME), featured by deficient oxid nitric (NO) formation, could be of interest to evaluate neural effects involved in the HPE[40], would be of interest to evaluate neural effects involved in the HPE.

Sedentary Lifestyle and gender are risck factors associated with cardiovascular diseases.[41,42] Only sedentary hypertensive animals were used in the selected studies. Senitko et al.[43] compared the influence of endurance exercise training status with sedentary normotensive individuals on the PEH. The falls in BP were by different mechanisms, increases on vasodilatation and reduction on cardiac output, respectively. Moreover, gender may have affected the response from cardiac autonomic regulation on PEH. Female SHR has higher

cardiac sympathetic tonus and HR and lower parasympathetic tonus at rest than males SHR[24,44]. After acute exercise, greater reduction in cardiac sympathetic tonus is found in females than males SHR[44]. Taken together, physical conditioning status and gender could cause PEH by different ways.

In this review, only one study investigated the resistance exercise-induced acute effects. The most studies of acute cardiovascular benefits from exercise in animal or human hypertensives are related to aerobic exercise.[12,18,45–47] However, recently resistance training also has been described as a safe and effective non-pharmacological tool for the treatment of cardiovascular diseases.[8,48–51] Most studies about hypertension, which investigate the post-resistance exercise hypotension, were performed in humans. Therefore, the deep understanding of mechanisms involved are limited.[52–54] Aerobic and resistance exercises provoke unique cardiovascular responses, consequently the mechanisms involved in PEH could be different.[13,17,25,31,55–57]

Regarding exercise intensity, all selected studies used were mild-to-moderate. The intensity and volume of exercise have been considered important variables in the exercise prescription for hypertensive and healthy individuals.[14,58] Although the exercise protocol used were sufficient to provide hypotension after exercise, the hemodynamic changes provocked by higher intensities or volumes of exercise could result in greater and more prolonged BP reductions.[15,56,59]

Not only the magnitude of PEH, but also its duration are important features to clinical relevance in the treatment of hypertension. In this review, the onset of hypotension following exercise has been found in a few minutes and remained throughout the monitoring time. Kajekar et al.[26] showed PEH in SHR up to 10 h after exercise.

Several researchers suggest different neural mechanisms involved in this hypotensive prolonged effect. Modulation of GABAergic system or increased expression of enkephalins in

the NTS[27,60], increased dopamine synthesis in the brain through a system dependent on calcium with activation of D2 receptor[28] and increased central vasopressin-1 receptor activation[29] are examples. In hypertensive humans, prolonged drop in BP were also observed in both aerobic and resistance exercise.[18,48,61] Therefore, in hypertensive humans the long duration of PEH have supported the use of exercise in its treatment.

The PEH in hypertensive animals presented several neural mechanisms involved. Such diversity of afferent[24,62–64], efferent[22,44,65] and central neural integration[23,26–29,60] pathways involved in this effect demonstrates its complexity in hypertensive animals. Among afferents mechanisms, responses from arterial baroreceptors demonstrated a crucial role in BP reduction after exercise. According to Chandler et al.[66], the sinoaortic denervation prevents the BP reduction as well as cardiac sympathetic tonus after exercise. Kajekar et al.[26] correlated the effect on arterial baroreflex gain with reduction of vasomotor tonus, which was provoked by the upregulation of GABA inhibitory signaling in the cardiovascular sympathetic neurons of RVLM. The reduction in neuronal output from RVLM contributes to the decrease in TPR.

Moreover, Minami et al.[64] demonstrated that hemodynamic changes during exercise, such as increased BP and tachycardia, are not the only afferent stimuli to increase arterial baroreflex sensitivity after exercise. Both cardiopulmonary and skeletal muscle afferents could change barosensitive neurons in the NTS, resulting in elevated NTS activity for any given arterial baroreceptor input. [24]

To supporting these evidences, it was shown that the NK1-R internalization of GABA inhibitory interneurons in NTS may influence the second-order neurons of the baroreceptors and consequently excite NTS after exercise.[15,23,27] This receptor internalization is produced by the accumulation of substance P that is released by skeletal muscle afferents stimulated during exercise. The NTS excitation is associated with sympathetic activity reduction of the RVLM[65] and therefore reduction in BP. Together, hemodynamic changes and activation of skeletal

muscle afferent, have fundamental role in increasing baroreflex gain during recovery time after exercise in SHRs.[64,67]

The reduction in BP without compensatory tachycardia or sympathetic activation, plus bradycardia in hypertensive animals after exercise has also been associated with involvement of the arterial baroreflex control of HR. The operating point of the baroreflex is not fixed and can be influenced by a variety of stimuli from peripheral or central nervous system. A possibility to explain this effect is that exercise resets the operating point of arterial baroreflex to lower levels of BP during recovery period, so that it operates around the new lower pressure and therefore contributes to maintenance of hypotension with or not bradycardia [24,64].

The PEH is produced by both the cardiac and vascular autonomic modulation.[22,65] The main efferent neural mechanisms involved are the reduction in cardiac[22] and vasomotor[65] sympathetic activity, which produce significant reduction in HR and peripheral vascular resistance, respectively. Furthermore, other peripheral mechanisms as reduced vascular responsiveness to α-adrenergic receptors has been observed after exercise[68], and therefore reducing the sensitivity to sympathetic vasoconstrictor stimulus. Although several studies have shown the involvement of the autonomic modulation in PEH after aerobic exercise in hypertensive animals[22,26,44,65], Lizardo et al.[25] have not found its involvement and attributed the hypotension to the increased bioavailability of nitric oxide, however was used resistance exercise protocol. Therefore, suggesting that PEH induced by aerobic and resistance exercise could be mediated by different mechanisms.

The NTS is the first site of sensory integration from peripheral cardiovascular receptors.[69] In addition, receives and sends projections to higher brain areas such as the hypothalamus.[69,70] Several studies have shown that functional or structural changes exercise-induced are presents in suprabulbar cardiovascular modulation areas.[70–72] Akiyama and Sutoo[28] demonstrated that acute exercise increases brain dopamine synthesis by calcium-dependent

pathway in periventricular regions of hypertensive animals. The increase in dopamine levels inhibit sympathetic nerve activity via dopamine D2-R and subsequently contributing to reduction in BP.[73] Furthermore, another study demonstrated that the arginine vasopressin-1 receptor antagonist in cerebral lateral ventricle attenuates PEH.[29] This effect may be due to AVP-induced facilitation in NTS and subsequent shifting in the operating point of the arterial baroreflex to a lower pressure. In this situation, AVP could augment the sympathoinhibition reflex and contribute, in part, to PEH.[74]

Supporting these data, others studies have suggested that projections oxytocinergic and vasopressinergic neurons from central command as paraventricular nucleus (PVN) converge to the NTS, RVLM, dorsal motor nucleus of the vagus (DMV) and nucleus ambiguus (NA), to coordinate complex cardiovascular adaptations during dynamic exercise.[70,75–77]

In hypertensive animals, low expression of oxytocin (OT) mRNA has been found in areas of the biosynthetic PVN (magnocellular and parvicellular) and low density of OT receptor in the NTS.[71,78,79] In normotensive animals, the release of OT in the NTS increases vagal discharge and increases bradycardia reflex through the facilitation of baroreflex control of HR.[80] Studies have shown that hypertensive trained rats increase OT mRNA expression in the PVN and dorsal brainstem (DBS=NTS+DMV) contributing to hypotension associated with bradycardia at rest.[71,78] To our knowledge there is no data showing whether this same mechanism is present after acute exercise in hypertensive rats.

CONCLUSION

In conclusion, the data reviewed here suggest that the complexity of neural network in the manifestation of PEH in hypertensive rats involves different neural mechanisms.

Nevertheless, the presence of a functional baroreflex, activation of skeletal muscle afferents and integration of bulbar areas have received great attention.

This framework can be understood according BP is a variable influenced by many factors and the effective contribution of each neural mechanism is not fully elucidated yet. Due to the complexity of integrating the neural network in the modulation of blood pressure further studies are needed to better understand this phenomenon, including the use of other exercise types.

Declaration of interest: The authors report no conflicts of interest.

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Figure and Tables

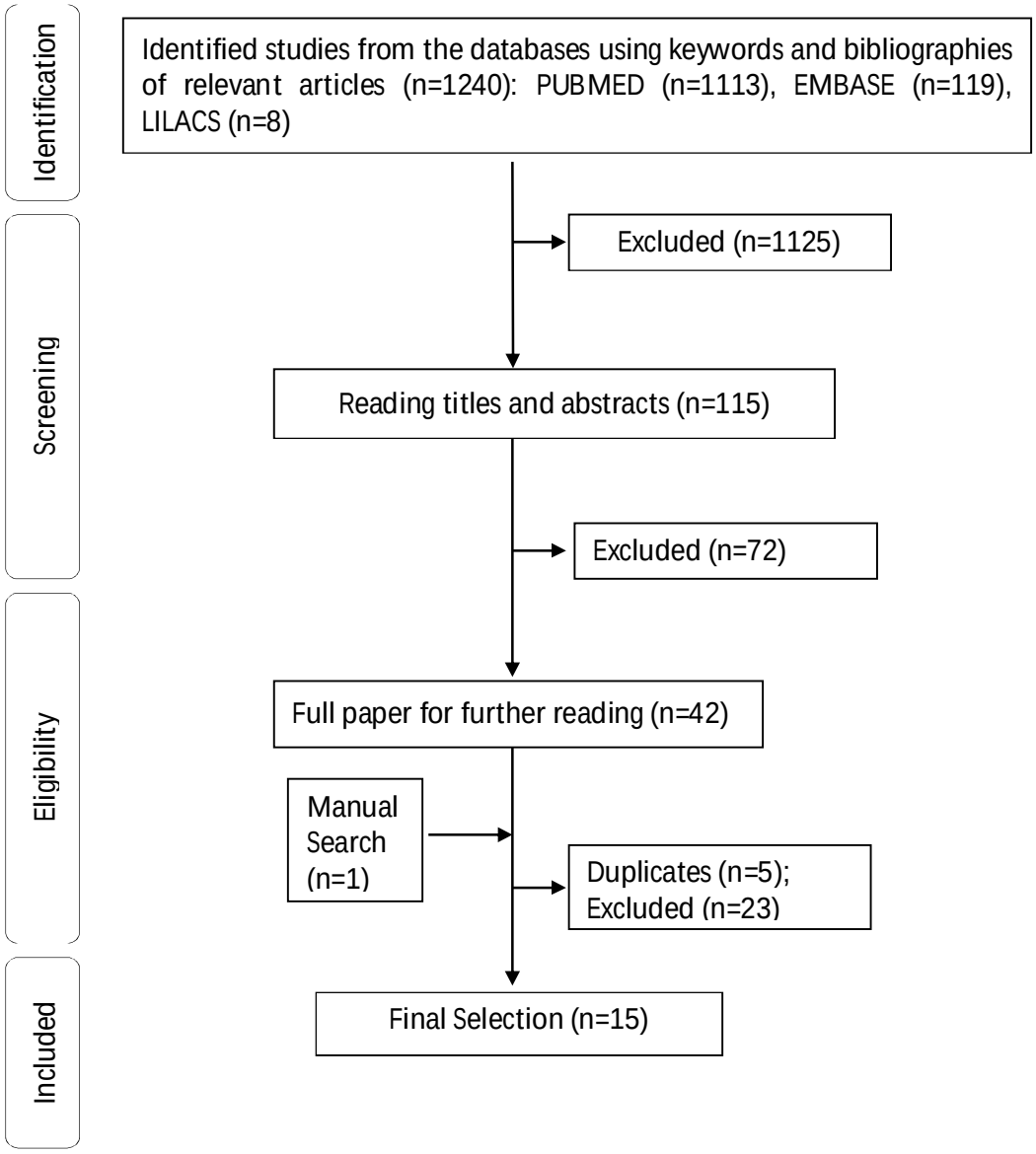

Figure 1. Flowchart of included studies

Table 1. Characteristics of included studies.

Authors, year, Country	Hypotheses	Animals and Hypertension models	Exercise			Protocol	Time of monitoring after exercise	Valued Parameter Settings		Results post-exercise and Neural Mechanisms
			Fitness level	Type	modality			Hemodinamics	Nervous system	
Collins HL and DiCarlo SE, 1993, USA	Cardiac afferents blockade would attenuate PEH	Male SHR	Sedentary	Aerobic	Motor-driven treadmill	9-12 m/min 10-18% grade 30-40 min	30 min	MAP and HR	Cardiac efferent and afferent	The fall in MAP was attenuated after blockade of afferent cardiac receptors, but not the efferents alone. There were no changes in HR in any trials. These data suggest that inhibitory influence of cardiac afferents may be enhanced after exercise.
Chen Y. *et al*, 1995, USA	Single bout of dynamic exercise decrease cardiac sympathetic tonus at rest	Male SHR	Sedentary	Aerobic	Motor-driven treadmill	12m/min, 10% grade 42 ± 1 min	20 min	MAP and HR	Cardiac Sympathetic (ST) and parasympathetic (PT) tonus	The drop in MAP was due to attenuation of the cardiac ST and PT. The reduction in HR, in the early recovery phase of exercise, was mediated by withdrawal of sympathetic tonus.
Boone Jr JB and Corry JM, 1996, USA	Acute exercise would increase proenkephalin mRNA in the NTS and RVLM.	Female, SHR 12 wk-old	Sedentary	Aerobic	Motor-driven treadmill	30 m/min 10% grade 40 min	30 min	MAP and HR	Proenkephalin mRNA expression at NTS, CVLM and RVLM	Reduction in MAP and HR and increase in proenkephalin gene expression in the NTS, CVLM and RVLM. These data suggest that increase in enkephalins synthesis and release may be involved in inhibitory influence on the bulbospinal simpathoexcitatory neurons after exercise.
Silva JJG. *et al*, 1997, Brazil	Acute exercise would increase the sensitivity of arterial baroreflex and CCB	Male SHR	Sedentary	Aerobic	Motor-driven treadmill	50% VO_2máx 45 min	30 min	MAP and HR	Arterial baroreflex and Chemiosensitive cardiopulmonary baroreflex	Acute exercise reduces MAP and increases arterial baroreflex bradycardia. This result suggest that the increases in baroreflex sensitivity may facilitate its control to inhibit increases in blood pressure at the recovery period of exercise.
Chandler MP and DiCarlo SE., 1997, USA	Arterial baroreflex is required for hypotension and sympathoinhibition that occurs after acute exercise	Male SHR, 13 wk-old	Sedentary	Aerobic	Motor-driven treadmill	12 m/min 10% grade 40 min	20 min	MAP, HR and HRi	Cardiac ST and PT	Sinoaortic denervation (SAD) prevented reductions in MAP and cardiac ST, but had no effect either HR or PT. This study demonstrate that the arterial baroreflex is required for PEH and cardiac sympathoinhibition after exercise.
Chandler MP and DiCarlo SE, 1998a, USA	Resting level of AP influence post-exercise cardiac autonomic responses	Male and female SHR 13 wk-old	Sedentary	Aerobic	Motor-driven treadmill	12 m/min 10% grade 40 min	60 min	MAP, HR and HRi	Cardiac ST and PT, Cardiac autonomic balance (CAB) and Relationship between HR and ST, PT and CAB	Reduction in MAP and HR in male and female in hypertensive rats, that was accompanied by a reduction ST, PT and CAB which demonstrated positive association between HR vs ST and CAB, but no PT. These results show that resting level of arterial pressure influence the autonomic regulation after exercise in SHR and that hypotension following bradycardia may be due increase on cardiac sympathoinhibition

Table 1. *Cont.*

Authors, year, Country	Hypotheses	Animals and Hypertesion model	Exercise			Protocol	Time of monitoring after exercise	Valued Parameter Settings		Results post-exercise and Neural mechanisms
			Fitness level	Type	Modality			Hemodinâmics	Nervous system	
Chandler MP. *et al.*, 1998b, USA	Post-exercise reductions in AP are mediated by lowering of the operating point of the arterial baroreflex	Male and Female SHR 13 wk-old	Sedentary	Aerobic	Motor-driven treadmill	12 m/min 10% grade 40 min	60 min	MAP and HR	Spontaneous baroreflex sensitivity (BRS)	Reduction in MAP, HR and gain of BRS in male and female rats. These results demonstrate that PEH accompanied of bradycardia may be associated to resetting the set point and reduction on gain of the arterial baroreflex control of HR after exercise.
Akiyama K and Sutoo D, 1999, Japan	Exercise may rectify hypertension trough affecting calcium and DA in the brain	Male SHR 12wks-old	Sedentary	Aerobic	Motor-driven wheel running	10 m/min 60 min	180 min	SBP (tail-cuff method)	Brain Calcium level, Both i.c.v. DA synthesis, D_1-R and D_2-R activation	Reduction in SBP and slow increase in brain calcium. Calcium-chelating agent, inhibitor of tyrosine hydroxylase and D_2-R antagonist, but no D_1-R antagonist, attenuated the reduction in SBP. These results suggest that hypotension caused by exercise occurs via D_2 R involved with calcium-dependent DA synthetized in the brain
Kulics JM. *et al*, 1999, USA	PEH is associated with reductions in TPR and SNA.	Male SHR 12wks-old	Sedentary	Aerobic	Motor-driven treadmill	12 m/min 10% grade 40 min	60 min	MAP, HR, CO and TPR	Lumbar Sympathetic Nerve Activity (LSNA)	Reduction in MAP, TPR and LSNA, increase in CO without changes in HR. These results suggest that PEH is associated with decrease in vasomotor SNA.
Collins HL. *et al*., 2001, USA	Central AVP mediates post-exercise reductions in MAP and HR.	SHR ~15wks-old	Sedentary	Aerobic	Motor-driven treadmill	12 m/min 10% grade 40 min	60 min	MAP, HR	Brain AVP V_1-R activation	Reduction in MAP and HR was prevented with central vasopressin V_1 receptor antagonist. This result suggest that vasopressin V_1 receptor activation has an important role in PEH.
Kajekar R. *et al*., 2002, USA	Baroreflex control and $GABA_A$-R in the RVLM contribute to PEH.	Male SHR 250-350 g	Sedentary	Aerobic	Motor-driven treadmill	15 m/min 10° grade 40 min	10h	MAP	Neurons sympathetic activity and $GABA_A$ receptors in RVLM and Baroreflex control of LSNA	Reduction in MAP, HR and activity from sympathetic cardiovascular neurons in the RVLM associated with significantly reduced LSNA and baroreflex gain. $GABA_A$ receptor antagonist increased the neurons activity from RVLM after exercise. This study suggest that upregulation of $GABA_A$ signaling in the RVLM neurons and reduced gain baroreflex may contribute to PEH by decrease in sympathetic outflow.
Chen CY. *et al*., 2002, USA	Substance P acting at NK-1 receptors in the NTS might contributes to PEH.	Male SHR 270-350 g	Sedentary	Aerobic	Motor-driven treadmill	15 m/min 10° grade 40 min	120 min	MAP and HR	Activation of NK-1 receptors in the NTS	Reduction in the peak and duration of MAP, but no HR, were found in spontaneous hypertensive rats with NK1-R antagonist into NTS after exercise. These data suggest that substance P (NK-1) receptor mechanism in the NTS contributes to PEH.

Table 1. *Cont.*

Authors, year, Country	Hypotheses	Animals and Hypertesion model	Exercise			Protocol	Time of monitoring after exercise	Valued Parameter Settings		Results post-exercise and Neural mechanisms
			Fitness level	Type	Modality			Hemodinamics	Nervous system	
Minami N. *et al*., 2006, Japan	Hemodinamic changes associated with dynamic exercise contribute to the post-exercise modulation of BRS	Male SHR 12 wks-old	Sedentary	Aerobic	Motor-driven treadmill	12 m/min 0°grade 40 min	30 min	MAP and HR	BRS and SBP-LFamp	Exercise associated with infusion of β and α adrenergic agonists provoked fall in MAP and HR, as well as increase in SBP-LFamp, however did not alter the baroreflex sensitivity after exercise. These results suggest that hemodynamic change during exercise alone does not contribute to the post-exercise increase of BRS and the augment of others afferent inputs may be improve the BRS after exercise.
Lizardo JHF. *et al*, 2008, Brazil	NO and ANS mediate PEH.	Male SHR 250-300g	Sedentary	Anaerobic	Squat exercise apparatus	10 sets 10 rep 70% of 1RM	120 min	MAP, SBP, DBP HR and DP	ANS	Reduction in MAP, SBP and DBP and increase in HR was elicited after exercise. Ganglionic blocker did not prevent the fall in MAP, but produced bradycardia, however the use of the inhibitor of NO synthase prevented the fall in MAP, SBP, DBP and increase in HR. These results suggest that NO, but no ANS, plays a crucial role in PEH.
Chen CY. *et al*., 2009, USA	Interaction between the substance P NK1-R and GABAergic transmission in the NTS may contribute to PEH	Male SHR, 12 wks-old	Sedentary	Aerobic	Motor-driven treadmill	15–16 m/min 10° grade 40 min	Not monitored	Not measured	sIPSC on NTS baroreceptor second-order neurons underwent to substance P perfusion with or without NK1-R antagonist mIPSC in the presence of TTX with or without NK1-R antagonist Triple-label for the NK1-R, GAD67 and Sytox green;	Reduction in the frequence of GABA sIPSC. Reduction in the endogenous and exogenous substance P influence on sIPSC frequency were mediated by a reduced responsiveness of NK1-R of inhibitory neurons. The NK1-R fluorescent intensity was overlapping labeling of GAD67. Taken together, these data suggest that exercise-induced internalization of NK1-R results in a reduced GABA inhibitory input to the neuron via the baroreflex. Arousal resulting from NTS causes PEH. .

ANS (autonomic nervous system); AP (arterial pressure); AVP (arginine vasopressin); BRS (baroreflex sensitivity); CAB (cardiac autonomic balance); CCB (Chemiosensitive cardiopulmonary baroreflex); CO (cardiac output); CVLM (caudal ventrolateral medulla); DA (dopamine); DP (double-product); $GABA_A$;(gamma-Aminobutyric acid receptor type A); GAD67 (glutamic acid decarboxylase 67); HR (heart rate); HRi (intrinsic heart rate); i.c.v. (intracerebroventricullar); L-NAME (N(G)-nitro-L-arginine methyl ester); LSNA (lumbar sympathetic nervous activity); MAP (mean arterial pressure); mIPSC (miniature spontaneous inhibitory post synaptic currents); NK1-R (Neurokinin type 1 receptor); NO (nitric oxide); NTS (Nucleus Tractus Solitarii); PEH (post-exercise hypotension); PT (parasympathetic tonus); RVLM (rostral ventrolateral medulla); SAD (sino aortic denervation); SBP (systolic blood pressure); SBP LFamp (systolic blood pressure-low frequency power amplitude); SHR (spontaneously hypertensive rat); sIPSC (spontaneous inhibitory post synaptic currents); SNA (sympathetic nervous activity); ST (sympathetic tonus); SYTOX green (nuclear counterstain); TPR (total peripheral resistance); TTX (tetrodotoxin); V_1-R (vasopressin type 1 receptor);

3.2 ATERIAL BAROREFLEX PARTICIPATES IN THE POST-RESISTANCE EXERCISE HYPOTENSION IN L-NAME-INDUCED HYPERTENSIVE RATS

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Arterial Baroreflex participates in the post-resistance exercise hypotension in L-NAME-induced hypertensive rats

Running Title: Baroreflex on post-resistance exercise hypotension

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Abreviation list

AH	Arterial hypertension
AVP	Arginine vasopressin
BRS	Barorreflex sensitivity
BP	Blood pressure
CAB	Cardiac autonomic balance
CO	Cardiac output
DAP	Diastolic arterial pressure
DBS	Dorsal brainstem
DMV	Dorsal motor nucleus of the vagus
EN	Exercised normotensive rats
EH	Exercised hypertensive rats
RE	Resistance exercise
GABA	Gamma-Aminobutyric acid
HF	High frequency component from pulse interval
HR	Heart rate
LF	Low frenquency component from pulse interval
LF/HF ratio	Low frequency/high frequency ratio
LFsys	Low frequency component from systolic arterial pressure
L-NAME	Nω-Nitro-L-arginine methyl ester
MAP	Mean arterial pressure
NK-1 R	Neurokinin 1 receptor
NTS	Núcleo do trato solitário
NO	Nitric Oxide
OT	Oxyitocina
OT mRNA	Oxytocin messenger Ribonucleic acid
OT-R	Oxytocin Receptor
PEH	Post-exercise hypotension
PI	Pulse interval
PVN	Paraventricular nucleus of hypothalamus
PVR	Peripheral vascular resistance
RE	Resistance exercise
RM	Repetition maximum

RVLM	rostral ventrolateral medulla nucleus
SAP	Systolic arterial pressure
SEM	Standard error mean
SHR	Spontaneously hypertensive rat
SH	Sham hypertensive animals
SN	Sham normotensive animals

Abstract

A single bout of exercise decreases blood pressure level in hypertensive with significant clinical relevance. This phenomenon is known as post-exercise hypotension (PEH), which has been induced by both aerobic and resistance exercise. However, probably neural mechanisms involved in resistance exercise is widely unclear. Therefore, the aim of this study was to verify hemodynamic changes and cardiovascular autonomic control during PEH after a single bout of resistance exercise in hypertensive rats. Were used wistar rats with Nω-Nitro-L-arginina metil éster (L-NAME)-induced hypertension (20 mg/kg daily). Cardiovascular evaluation was performed in conscious animals during 30 min before and 2 hours after exercise protocol, which consisted of 10 sets of 10 repetitions with 2 min of rest interval and performed at 60% of one repetition maximum test in squat-training apparatus. Spontaneously Baroreflex sensitivity (BRS) was analyzed by sequence method and cardiac autonomic balance by heart rate variability in the frequency domain. A single bout of resistance exercise was able to induce PEH (Mean arterial pressure: from 159.7 ± 3.1 to 144.7 ± 2.7 mmHg, $p < 0.01$) followed by bradycardia (Hert rate: from 361.7 ± 9.7 to 310.8 ± 13.0 bpm, $p < 0.05$), increase BRS (from 1.0 ± 0.2 to 2.8 ± 0.5 mmHg/s, $p<0.05$) and reduce cardiac (Low Frequency/High Frequency ratio: from 0.35 ± 0.04 to 0.24 ± 0.02 $p<0.05$) and vascular (Low Frequency systolic: from 5.25 ± 0.5 to 3.54 ± 0.3, $p<0.05$) sympathetic modulation in in L-NAME-induced hypertensive rats. Together, our results suggest that the baroreflex plays a important role in the development of PEH, probably increasing sympathetic inhibition on heart and vessel.

Keywords: hypertension, post-exercise hypotension, exercise, physical conditioning, animal, autonomic nervous system, central nervous system.

INTRODUCTION

Hypertension is usually defined by the presence of a chronic elevation of systemic arterial pressure above a certain threshold value (Giles et al., 2009, 2005; Mancia et al., 2013). It is a disease that already affects one billion people worldwide, leading to heart attacks and strokes (WHO, 2013).

Accumulating evidence indicates that central activation of the sympathetic nervous system plays an important role in hypertension (Coffman, 2011; Esler, 2011, 2010; Grassi, 2010, 2009; Grassi et al., 2010; Guyenet, 2006; Malpas, 2010). Important studies revealed that arterial baroreflex has a role in long-term blood pressure (BP) regulation (Iliescu et al., 2012; Lohmeier and Iliescu, 2011; Thrasher, 2005). Studies have demonstrated abnormalities of baroreceptor function, but it is unclear why enhanced sympathetic activity occurs in hypertension. These findings have attracted the interest of many researchers in the brain mechanisms leading to enhanced central sympathetic outflow in hypertension (Chan and Chan, 2012; Fisher and Fadel, 2010; Gabor and Leenen, 2012; Guyenet, 2006; Hirooka et al., 2011).

Regarding the therapy of hypertension, after decades of improvement in its control with pharmacological approaches, only 31% adults individuals are adequately controlled in the United States (Lloyd-Jones et al., 2009). This downward trend in pharmacological management has led to renewed efforts to reduce the prevalence of hypertension with nonpharmacological approaches (Appel, 1999). In this context, exercise training is also widely recommended for decreasing BP (Pescatello et al., 2004) and reducing cardiovascular mortality (Engström et al., 1999; Taylor et al., 2006; Wisløff et al., 2006).

Recently attention has been given not only chronic effects from exercise, but also to the effects from a single bout of exercise (Halliwill et al., 2013; Hamer, 2006). After exercise, BP levels decrease within minutes and persist for hours in relation to pre-exercise levels (Brandão

Rondon et al., 2002; Halliwill, 2001; Kenney and Seals, 1993; MacDonald, 2002; Melo et al., 2006; Mota et al., 2009; Queiroz et al., 2013, 2009; Rezk et al., 2006). This phenomenon is known as post-exercise hypotension (PEH) and has been widely investigated, because it is of great clinical relevance for the treatment and prevention of arterial hypertension (AH) (Halliwill, 2001; Kenney and Seals, 1993; MacDonald, 2002; Pescatello et al., 2004).

PEH is associated with a sustained reduction in peripheral vascular resistance (PVR) and a rise in systemic vascular conductance (Halliwill et al., 1996a). The post-exercise decrease in PVR may be due to the reduction of sympathetic nerve activity in the autonomic nervous system (Floras et al., 1989; Halliwill et al., 1996a; Kulics et al., 1999), as well as decreased vascular responsiveness to α-adrenoceptor activation (Rao et al., 2002).

The understanding of possible neural mechanisms involved in PEH is from studies with aerobic exercise. Although PEH in hypertensive humans have also been demonstrated after resistance exercise (RE) (Brito et al., 2011; Melo et al., 2006; Moraes et al., 2012; Mota et al., 2013), possible neural mechanisms involved are unclear. An understanding of the mechanisms underlying PEH may be the first step in designing strategies to control AH, allowing greater emphasis on physical exercise, especially acute RE (Lizardo et al., 2008). Therefore, the purpose of this study was verify hemodynamic changes and cardiovascular autonomic control during PEH after a single bout of moderate RE in L-NAME-induced hypertensive rats.

METHODS

Animals

Experiments were performed in male *Wistar* rats weighing between 250 and 300 g. The animals were housed in individual cages with free access to water and food, at a constant temperature of 22 ± 1ºC, on a 12 h light/dark cycle. All experimental protocols were in

accordance with the Guidelines for Ethical Care of Experimental Animals and were approved by the Animal Research Ethics Committee of the Federal University of Sergipe (São Cristovão, SE, Brazil #87/2013).

Hypertension induction

To obtain L-NAME-induced hypertension (Sigma-Aldrich, St. Louis, MO, USA), male *Wistar* rats were treated orally by gavage with L-NAME (20 mg/kg, daily) for 7 days as described by Biancardi et al. (2007). Normotensive rats underwent the same manipulation daily by oral gavage using only vehicle and were used as control.

Surgical procedure

Surgical instrumentation was performed using aseptic surgical procedures. The animals were anesthetized with thiopental sodium (45 mg/kg, i.p.) and right carotid artery was carefully isolated to avoid damage to any nearby nerves. Polyethylene catheter was implanted (PE-50, Intramedic, Becton Dickinson and Company, Sparks, MD, USA) into the right common carotid artery for measurements of BP and heart rate (HR). The catheter was filled with heparinized saline (1:9 mL), its free end plugged with a stainless steel obturator and tunneled subcutaneously to exit from the back of the neck and surgical incision sutured. Rats were then placed in separated cages and allowed to recover for 24 hours before experimentation.

Cardiovascular assessment

Conscious rats were studied 24 hours after surgical procedure and allowed to move freely during the experiments. On the day of experiments, rats were allowed to adapt to the laboratory environment for 1 h before obtain resting hemodynamic measures. The arterial catheter was connected to a pressure transducer (Edwards Lifescience, Irvine, CA, USA) and

coupled to a preamplifier (BioData, Model BD-01, PB, Brazil). The pulsatile arterial pressure was recorded in an IBM/PC with analog-to-digital interface (2 kHz; BioData, BD, Brazil). The AP signal was processed by computer software (Advanced Codas/Windaq, Dataq Instruments Inc., Akron, OH, USA), inflection points were identified and the signal generated time series beat-to-beat. Values of mean arterial pressure (MAP), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), pulse interval (PI) and HR were obtained and assessed during 30 min before and 120 min after exercise. Average periods of 15 minutes were analyzed.

Autonomic control

The baroreflex sensitivity (BRS) was measured in the time domain by the sequence method (Bertinieri et al., 1985). Series beat-to-beat were analyzed by software CardioSeries v2.4. Sequences of at least 4 heartbeats with increased SAP followed by PI lengthening or subsequent decrease of SAP with PI shortening with correlation greater than 0.85 were identified as baroreflex sequence. The slope of the linear regression between PAS and PI was considered as a measure of BRS.

Cardiac autonomic balance was evaluated by frequency domain. The PI and SAP variability analysis was performed by software CardioSeries v.2.4 (http://sites.google.com/site/cardioseries). Series beat-to-beat was obtained by pulsatile AP and converted into points every 100 ms using cubic spline interpolation (10 Hz). The interpolated series was divided into half-overlapping sequential sets of 512 data points (51.2 s). Before calculation of the spectral power density, the segments were visually inspected and the nonstationary data were not taken into consideration. The spectrum was calculated from the Fats Fourier Transfomation (FFT) algorithm direct and Hanning window was used to attenuate side effects. The spectrum is composed of bands of low frequency (LF; 0.2-0.75 Hz) and high frequency (HF; 0.75-3 Hz), the results were showed in normalized units, by calculating the

percentage of the LF and HF variability with respect to the total power after subtracting the power of the Very low frequence (VLF) component (frequencies<0.20 Hz), namely Low Frequency/High Frequency (LF/HF) ratio.

The LF/HF ratio from interval pulse represents sympathovagal balance. LF and HF components mean cardiac sympathetic and parasympathetic activity. LF from systolic arterial pressure (LFsys) represents sympathetic vascular modulation ("Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology," 1996; Montano et al., 1994).

Exercise protocol

Animals performed RE according to a model described by Tamaki et al. (1992) that simulates squat-training in humans. Rats wearing a canvas jacket were able to regulate the twisting and flexion of their torsos and were fixed by the holder in a standing position on their hindlimbs. The animals were stimulated to perform sets of exercise by an electrode on the tail connected to an electrical stimulator (BIOSET, Physiotonus four, Model 3050, Rio Claro, São Paulo, Brazil). The parameters used were: 1 Hz frequency, pulse width of 1 ms, time on 1-3 seconds and time off 2 seconds and intensity enough that the animals perform physical exercises, ranging from 4 to 15 mA.

Before RE itself, the rats were submitted to a 1 week adaptation program in the exercise apparatus. Two days before experimentation all groups were subjected to the 1 repetition maximum test (1RM) for determining the exercise workload and then underwent the surgery for arterial catheterization. The 1RM test was determined as the maximum weight lifted with the exercise apparatus in unique repetition (Barauna et al., 2005).

Before beginning exercise experiments, normotensive and hypertensive animals were randomized in sham normotensive (SN) (n=5) or exercised normotensive (EN) (n=5) and sham hypertensive (SH) (n=5) or exercised hypertensive (EH) (n=5) groups, respectively.

RE was performed with 10 sets of 10 repetitions. The repetitions were performed at 2 s intervals with a 2 min rest period between sets. The exercise intensity was 60% of 1 RM. The Sham animals underwent a fictitious exercise. In the exercise apparatus Sham rats, received electrical stimulation on the tail at intervals and intensity similar to exercised animals, however the equipment had no resistance and was maintained in the rest position, preventing thus the implementation effort.

Statistical analysis

Values are expressed as mean ± standard error mean (SEM). Unpaired Student's test was used to compare significant differences in baseline values from normotensives vs hypertensive animals. Two way ANOVA followed by Bonferroni post-test was used in order to evaluate the significance of differences intragroup and their controls. $p < 0.05$ was considered significant. All statistical analyses were done by using Graph Pad Prism TM version 5.0 software.

RESULTS

Table 1 presents body weight and maximal workload lifted by each group. No significant differences were found between groups.

Hemodynamic parameters from all groups at rest (baseline) and during 2 hours after exercise were expressed in figure 1. Hypertensive animals presented higher MAP ($p<0.001$),

SAP ($p<0.001$) and DAP ($p<0.001$) at rest when compared to normotensive animals. However, no change in HR at rest was found between hypertensive and normotensive ($p>0.05$).

In both exercised groups, EH and EN, showed a biphasic response from MAP when compared to their baselines (figure 1A). MAP increased immediately post-exercise up to 15 minutes in EH ($p<0.01$) and EN ($p<0.001$) and followed hypotension period after 45 min in EH ($p<0.05$) and 60 min in EN ($p<0.05$) which persisted all period recovery.

Concerning SAP (figure 2B) when compared their baselines, EH and EN increased immediately post-exercise up to 15 minutes ($p<0.01$ and $p<0.001$, respectively). However, lowering of SAP was observed only in EH which started after 45 min ($p<0.01$) and persisted all period recovery after exercise. In EN, SAP returned to its baseline value 15 minutes post-exercise and remained as well throughout the recovery period.

DAP (figure 1C) showed similar biphasic response only in EN group, which demonstrated increase post-exercise up to 15 minutes ($p<0.01$). Following this, in both EH and EN, DAP decreased after 45 min post-exercise when compared to their baselines ($p<0.05$ and $p<0.001$, respectively) which persisted all exercise recovery time.

A single bout of moderate RE produced bradycardia (figure 1D) post-exercise only in EH, when compared to its baseline ($p<0.05$) during 75-120 min of recovery period after exercise.

Spontaneous BRS of all groups at rest and after exercise was expressed in figure 2. Hypertensive animals presented lower BRS at rest when compared with normotensive animals ($p<0.05$). Immediately after exercise, both EH and EN, increased BRS when compared to their controls or baseline ($p<0.05$ and $p<0.001$, respectively) and persisted up to 105 min after exercise.

LF/HF ratio and LFsys of all groups at rest and after exercise were expressed in figure 3A and 3B, respectively. Hypertensive animals presented higher LF/HF ratio ($p<0.01$) and

LFsys ($p<0.01$) at rest when compared with normotensive animals. Immediately after exercise up to 15 min LF/HF ratio was increased in both, EH ($p<0.01$) and EN ($p<0.01$), when compared to their baselines. However only EH animals showed significantly decrease in LF/HF ratio after 45 min ($p<0.05$) when compared to SH or its baseline which persisted lower all long recovery period.

In addition, a single bout of RE was able to reduce LFsys in both EH after 15 min ($p<0.05$) and EN after 30 min ($p<0.01$) when compared to their baselines, which also persisted during all recovery period.

DISCUSSION

In this study the effects of a single bout of moderate RE were evaluated on the hemodynamic response and autonomic function in L-NAME-induced hypertensive rats. To our knowledge, this is the first study that found post-resistance exercise hypotension (PREH) in L-NAME-induced hypertension model. In this study PREH following by bradycardia was mediated by increased in baroreflex sensitivity which reduced cardiac and vascular sympathetic modulation.

In L-NAME-induced hypertension, there is altered balance between the enhanced sympathetic vasoconstriction and the attenuated vasodilatation (due to missing nitric oxide, NO, and insufficiently up-regulated endothelium-derived hyperpolarizing factor) (Kunes et al., 2004; Pechánová et al., 2004; Török, 2008), increase in plasma noradrenaline and adrenaline levels and activation of renin-angiotensin system (Zanchi et al., 1995). NO deficiency in the central nervous system contributes to cardiovascular disorders as decrease baroreflex sensitivity and produce overactivity from rostral ventrolateral medulla nucleus (RVLM) neurons, an

important source of sympathetic cardiovascular activity (Bergamaschi et al., 1999; Biancardi et al., 2007; Gerová et al., 1995; Souza et al., 2001).

Our study also found cardiovascular autonomic dysfunction after orally chronic 7-days treatment with the NO synthase inhibitor, L-NAME. This effect was observed in the pre-exercise period by reduction of baroreflex sensitivity, increased cardiac sympathetic participation by LF/HF ratio and increased vasomotor tone by LFsys. Therefore, this is an appropriate hypertension model to evaluate the participation of neural mechanisms possibly involved in this study (Souza et al., 2001).

Immediately after exercise up to 15 min, MAP was elevated due to isolated increase in SAP of EH group, then returned to baseline values. During exercise the cardiac output (CO) increases significantly to ensure adequate perfusion to the exercising muscles (Boushel, 2010; MacDonald, 2002). The muscle metabolic demands created during exercise persist minutes after exercise and therefore may maintain high CO (Cléroux et al., 1992; MacDonald, 2002), which in part may be attributed to increased BP. In our study SAP remained high due to increased cardiac sympathetic activity observed by elevated LH/HF ratio. No change in PVR was found in the same period verified by sympathetic vasomotor modulation in LFsys. However, the likely participation of local vasodilators mechanisms (Halliwill et al., 2013) should be considered since it was found no increase in DAP, which reflects the total peripheral resistance.

The occurrence of PREH is controversial (Rezk et al., 2006). Furthermore, most studies investigating PREH in hypertension were performed in humans and understanding the mechanisms involved in this effect is not clear (Fisher, 2001; Hardy and Tucker, 1998; Melo et al., 2006; Moraes et al., 2012).

In this study, the hypotension in EH occurred by simultaneous and proportional decrease in SAP and DAP. Another study that used RE found similar results with a higher participation

of SAP decrease in the hypotensive effect (Lizardo et al., 2008). In hypertensive humans both SAP and DAP reduction also have been found after RE (Melo et al., 2006; Moraes et al., 2007). The drop in SAP in this study may have been caused by the reduction in sympathetic cardiac activity seen by LF/HF ratio.

Sustained reduction of PVR and rise in systemic vascular conductance during PEH contribute to decrease DAP (Halliwill et al., 1996a). In our study, the reduction in DAP involved, at least in part, decreased sympathetic vascular modulation observed by falling in LFsys.

Currently our group has shown that resistance training is effective in the control of MAP and DAP in L-NAME-induced hypertension model. These effects were attributed to attenuation of local vasoconstrictor mechanisms and maintenance of the luminal diameter (Araujo et al., 2013). Another study from our group showed that acute RE promotes enhanced insulin-induced vasodilation, however in normotensive animals (Fontes et al., 2014). Supporting these data, Faria et al. (2010) and Lizardo et al. (2008) also showed endothelium-dependent relaxation in spontaneously hypertensive animals (SHR) after RE.

The occurrence of hypotension after exercise in this study, even with a significant decrease in the local vasodilator mechanism, suggests that this effect may have been mediated, at least in part, by neural factors as well as it has been observed in other studies with aerobic exercise in humans and animals. Some effects from aerobic exercise involve: increase in arterial baroreceptors sensitivity (Convertino and Adams, 1991; Halliwill et al., 1996b; Minami et al., 2006; Silva et al., 1997; Somers et al., 1985); activation of cardiac afferents (Collins and DiCarlo, 1993); reduction cardiac sympathetic tone (Chandler and DiCarlo, 1998; Chen et al., 1995); activation of substance P receptor (Neurokinin-1) in the nucleus tractus solitarii (NTS) (Chen et al., 2002); increase in GABAergic inhibition on RVLM and vasomotor tone (Kajekar et al., 2002; Kulics et al., 1999); involvement of opioid mechanisms on NTS and RVLM (Boone

and Corry, 1996) and increased central vasopressin and activation of V_1 vasopressin receptors (Collins et al., 2001).

To our knowledge this is the first study that links the participation of arterial baroreflex sensitivity in PREH to L-NAME-induced hypertension. The increased BRS observed in this study, may have reduced cardiac and vascular sympathetic modulation verified by LF/HF ratio and LFsys reduction respectively, suggesting its participation in hypotensive and bradycardic effect.

Supporting these data, the reduction in BP without a baroreflex-mediated compensatory tachycardia or even bradycardia as observed in our study, suggests that a single bout of RE may resets the operating point of the arterial baroreflex to a lower pressure so that it now operates around the new lower pressure as seen in aerobic exercise (Chandler et al., 1998). In addittion, the baroreflex is less sensitive to falling blood pressure than to rising BP (Willie et al., 2011). In another study, sinoaortic denervation in SHR prevented the PEH and reduction in cardiac sympathic tone, which demonstrated the importance of the arterial baroreflex in the hypotensive response after exercise aerobic exercise (Chandler and DiCarlo, 1997). Concomitant decrease in arterial pressure and sympathetic nerve activity have been explained using the concept of acute resetting of baroreflex control of sympathetic nerve activity (Miki et al., 2003).

Other studies have demonstrated the involvement of muscle (Chen et al., 2002) and cardiac (Collins and DiCarlo, 1993) afferents in the manifestation of PEH, suggesting that there are some interactions in the neural networks regulating exercise and BP. The NTS is the first central site of integration of cardiovascular sensory information coming from the periphery, its excitation decrease BP by modulation the response of other nucleus in autonomic cardiovascular control, in particular on RVLM, which is a important site of origin of the sympathetic cardiovascular tone (Chen and Bonham, 2010; Dampney, 1994).

In hypertensive animals, there is an increase in tonic inhibition of GABAergic interneurons in NTS which hinders modulation from arterial baroreceptors afferent to decrease sympathetic activity (Mei et al., 2003; Zhang and Mifflin, 2010). Studies, in hypertensive rats, showed that a single bout of aerobic exercise was able to induce tonic reduction of GABAergic activity in the NTS through the internalization of NK1-R from substance P present in these interneurons and stimulated by skeletal muscle afferent during exercise (Chen et al., 2009, 2002). Therefore, allowing the baroreflex response excite the NTS then provide a tonic inhibitory input to sympathetic premotor neurons in the RVLM.

Furthermore, reciprocal projections of NTS-PVN-NTS may be involved in the cardiovascular modulation during exercise or after training especially in the HR reflex control (Michelini and Stern, 2009). Projections of oxytocinergics (OTergics) pre-autonomic neurons from paraventricular nucleus (PVN) to NTS and dorsal motor nucleus of the vagus (DMV) may participate in the modulation of bradycardia reflex facilitation inhibition of RVLM and exciting vagal tone of the heart, respectively (Higa et al., 2002; Higa-Taniguchi et al., 2009).

However in hypertensive rats there are both reduction in OT-R density in NTS as OT mRNA expression in PVN (Martins et al., 2005). In this same study was demonstrated that exercise training increased the OT mRNA expression on PVN and DBS areas in SHR. In another study, sinoaortic denervation abolished PVN OT mRNA expression and reduced PVN OT density in SHR (Cavalleri et al., 2011), showing the importance of baroreceptors in this response. No data regarding RE was found. The bradycardia found in our study could have been caused by improved OTergic system modulated by NTS-PVN-NTS network, which decreases sympathetic cardiac autonomic modulation.

Thus, the neural mechanisms involved in post-resistance exercise hypotension followed by bradycardia, could involve both ascending pathways from muscle and visceral receptors as well as descendants pathways from hypothalamus, however sharing in common the resetting of

operating point from arterial baroreceptor to lower blood pressure, which reduces vasomotor and cardiac sympathetic modulation.

CONCLUSION

Taken together, our data showed that RE promotes post-exercise hypotension following by bradycardia in L-NAME-induced hypertension through cardiac and vascular sympathetic activity reduction mediated by increases in BRS. However, given the many elements that regulate blood pressure and interaction between these factors, may be difficult to identify a single causal mechanism. Therefore, more studies should be done to explain the PREH. In addition, the effects observed in this study support the use of moderate RE to non-pharmacological treatment of hypertension, which has been shown safe and effective.

Study limitations:

Direct measure of the sympathetic nerves activity by microneurography and cardiac output as well as labeling bulbar cardiovascular areas by immunofluorescence are important tools to confirm these possible neural pathways involved in PREH in hypertensive rats.

Declaration of interest: The authors report no conflicts of interest.

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Figures and Tables

Figure 1.

Figure 2.

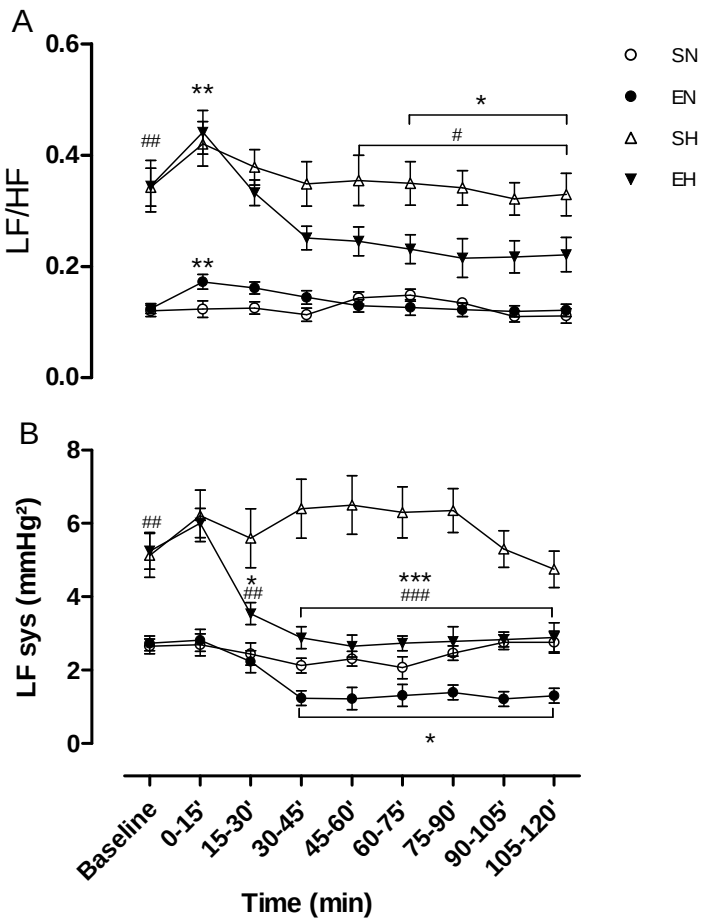
A
LF/HF
0.6
0.4
0.2
0.0
SN
EN
SH
EH
B
LF sys (mmHg²)
8
6
4
2
0
Baseline
0-15'
15-30'
30-45'
45-60'
60-75'
75-90'
90-105'
105-120'
Time (min)

Figure 3.

Table 1.

	SN (n = 5)	EN (n = 5)	SH (n = 5)	EH (n = 5)
Body weight (g)	284 ± 2.5	290 ± 4.9	286 ± 4.6	282 ± 5.3
1RM test (g)	1166 ± 46	1200 ± 40	1100 ± 63	1133 ± 61

SN: Sham normotensive; EN: Exercised normotensive; SH: Sham hypertensive; EH: Exercised hypertensive; 1RM test: 1 Repetition maximum test. Data are presented as means ± SEM. To evaluate difference between groups, it was used one-way ANOVA test followed by Bonferroni post-test.

Figure legends

Figure 1. Effect of a single bout of moderate resistance exercise on hemodynamic parameters in L-NAME-induced hypertensive rats. A: Mean Arterial Pressure (MAP), B: Systolic Arterial Pressure (SAP), C: Diastolic Arterial Pressure (DAP) and D: Heart Rate (HR) before and after resistance exercise in Sham Normotensive (SN), Exercised Normotensive (EN), Sham Hypertensive (SH) and Exercised Hypertensive (EH) animals. Data are presented as means ± SEM. To evaluate difference between groups, it were used unpaired t-test or two-way ANOVA followed by Bonferroni post-test. *$p<0.05$; **$p<0.01$; ***$p<0.001$ when compared each group with their baseline and $^{\#}p<0.05$; $^{\#\#}p<0.01$; $^{\#\#\#}p<0.001$ when compared EN *vs* SN or EH *vs* SH.

Figure 2. Effect of a single bout of moderate resistance exercise on spontaneous braroreflex sensitivity (BRS) in L-NAME-induced hypertensive rats. BRS was evaluated before and after resistance exercise in Sham Normotensive (SN), Exercised Normotensive (EN), Sham Hypertensive (SH) and Exercised Hypertensive (EH) animals. Data are presented as means ± SEM. To evaluate difference between groups, it were used unpaired t-test or two-way ANOVA followed by Bonferroni post-test. *$p<0.05$; **$p<0.01$ when compared each group with their baseline and $^{\#}p<0.05$; $^{\#\#}p<0.01$; $^{\#\#\#}p<0.001$ when compared EN *vs* SN or EH *vs* SH.

Figure 3. Effect of a single bout of moderate resistance exercise on cardiac autonomic balance (CAB) and low frequency component (LFsys) in L-NAME-induced hypertensive rats. CAB was evaluated by A: LF/HF ratio from interval pulse and B: LFsys from systolic arterial pressure before and after resistance exercise in Sham Normotensive (SN), Exercised Normotensive (EN), Sham Hypertensive (SH) and Exercised Hypertensive (EH) animals. LF = Low frequency; HF = High frequency. Data are presented as means ± SEM. To evaluate

difference between groups, it were used it was used unpaired t-test or two-way ANOVA followed by Bonferroni post-test. *$p<0.05$; **$p<0.01$; ***$p<0.001$ when compared each group with their baseline and $^{\#}p<0.05$; $^{\#\#}p<0.01$; $^{\#\#\#}p<0.001$ when compared EN *vs* SN or EH *vs* SH.

Table legends

Table 1. Body weight and 1RM test peformed 1 day before the single bout of moderate resistance exercise in L-NAME-induced hypertensive rats.

CONCLUSÃO

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A partir da análise dos artigos encontrados na literatura científica acerca dos mecanismos neurais envolvidos na hipotensão pós-exercício de animais hipertensos, pode-se concluir que diversas vias contribuem para a sua completa manifestação em ratos hipertensos. No entanto especial atenção é dada a participação do barorreflexo arterial, ativação de aferência muscular esquelética durante o exercício, além de prováveis modulações cardiovasculares tanto bulbares quanto suprabulbares com consequente redução da atividade simpática cardíaca e vascular.

Embora a grande maioria dos estudos em animais hipertensos que observaram a HPE tenham utilizado protocolos de exercício aeróbico foi possível verificar no presente estudo que o exercício resistido de intensidade moderada é capaz de produzir significativa HPE seguida de bradicardia em animais hipertensos induzidos por L-NAME. Tal queda sustentada na pressão arterial média foi decorrente de redução tanto na pressão arterial sistólica quanto diastólica sugerindo desta forma prováveis efeitos cardíacos e vasculares.

Quanto aos mecanismos neurais envolvidos na hipotensão pós-exercício resistido foi observado aumento da sensibilidade do barorreflexo arterial e redução da modulação simpática cardíaca e vascular. Além disso, a associação da hipotensão com bradicardia observada neste estudo sugere que o barorreflexo tenha reiniciado seu ponto de operação para níveis mais baixos de pressão arterial.

Por conseguinte os efeitos agudos cardiovasculares decorrentes do exercício resistido de intensidade moderada em ratos hipertensos em parte são mediados pelo aumento da sensibilidade barorreflexa e que seu uso se demonstrou seguro e eficaz para o tratamento da hipertensão. No entanto mais estudos são necessários para melhor esclarecer mecanismos centrais de integração envolvidos nestes efeitos.

PERSPECTIVAS

5 PERSPECTIVAS

Como o presente estudo não utilizou abordagens diretas de medida da atividade nervosa simpática, nem investigou o envolvimento de núcleos de controle cardiovasculares centrais, as próximas etapas devem investigar, através das técnicas de microneurografia, imunofluorescência e PCR T (*real time polymerase chain reaction*), alterações funcionais nos mecanismos neurais de controle da pressão arterial induzidas pelo exercício resistido agudo. Tais como: Amplitude e frequência de disparo do nervo simpático lombar para verificar a redução da resistência vascular periférica; marcação de proteína c-FOS no NTS, CVLM, RVLM, PVN e SON; Além de expressão de RNAm da OT no PVN e RNAm do OT-R no NTS para identificar os núcleos estimulados pelo exercício resistido na hipotensão seguida de bradicardia em animais hipertensos.

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ANEXOS

ANEXO A – Artigo publicado no *Life Sciences 94 (2014) 24–29* "Resistance exercise acutely enhances mesenteric artery insulin-induced relaxation in healthy rats"

Life Sciences 94 (2014) 24–29

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Resistance exercise acutely enhances mesenteric artery insulin-induced relaxation in healthy rats

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ABSTRACT

Aims: We evaluated the mechanisms involved in insulin-induced vasodilatation after acute resistance exercise in healthy rats.
Main methods: Wistar rats were divided into 3 groups: control (CT), electrically stimulated (ES) and resistance exercise (RE). Immediately after acute RE (15 sets with 10 repetitions at 70% of maximal intensity), the animals were sacrificed and rings of mesenteric artery were mounted in an isometric system. After this, concentration–response curves to insulin were performed in control condition and in the presence of LY294002 (PI3K inhibitor), L-NAME (NOS inhibitor), L-NAME + TEA (K^+ channels inhibitor), LY294002 + BQ123 (ET-A antagonist) or ouabain (Na^+/K^+ ATPase inhibitor).
Key findings: Acute RE increased insulin-induced vasorelaxation as compared to control (CT: $R_{max} = 7.3 \pm 0.4\%$ and RE: $R_{max} = 15.8 \pm 0.8\%$; $p < 0.001$). NOS inhibition reduced ($p < 0.001$) this vasorelaxation from both groups (CT: $R_{max} = 2.0 \pm 0.3\%$, and RE: $R_{max} = -1.2 \pm 0.1\%$), while PI3K inhibition abolished the vasorelaxation in CT ($R_{max} = -0.1 \pm 0.3\%$, $p < 0.001$), and caused vasoconstriction in RE ($R_{max} = -6.5 \pm 0.6\%$). That insulin-induced vasoconstriction on PI3K inhibition was abolished ($p < 0.001$) by the ET-A antagonist ($R_{max} = 2.9 \pm 0.4\%$). Additionally, acute RE enhanced ($p < 0.001$) the functional activity of the ouabain-sensitive Na^+/K^+ ATPase activity ($R_{max} = 10.7 \pm 0.4\%$) and of the K^+ channels ($R_{max} = -6.1 \pm 0.5\%$; $p < 0.001$) in the insulin-induced vasorelaxation as compared to CT.
Significance: Such results suggest that acute RE promotes enhanced insulin-induced vasodilatation, which could act as a fine tuning to vascular tone.

Introduction

Several authors have demonstrated the ability of exercise to prevent cardiovascular risk factors, among them endothelial dysfunction (Di Francescomarino et al., 2009; Golbidi and Laher, in press; Green et al., 2004; Zanesco and Antunes, 2007). The literature has demonstrated the ability of both chronic and acute aerobic exercise to improve the insulin signaling pathway involved not only in the glucose metabolism but also in the vascular modulation (Caponi et al., 2013; Pauli et al., 2010; Yang et al., 2006, 2010). In particular, resistance exercise has been also used for improvement of diabetes, hypertension and obesity (Westcott, 2012). Nevertheless, the signaling pathways are not clear.

Hemodynamic effects of insulin occur for two different endothelium-dependent signaling pathways: IR/PI3K/eNOS, responsible for the relaxant effect, and IR/MAPK/ET-1, responsible for the contractile effect (Chaudhuri et al., 2012; Montagnani et al., 2001; Muniyappa and Quon, 2007; Salt, 2013). Thus, the balance between the release of NO and ET-1 plays an important role for the control of vascular tone and blood flow adjustments in response to the exercise (Mather et al., 2001; Muniyappa and Sowers., 2013).

Previous studies have shown that insulin-induced vasorelaxation is enhanced in animals after aerobic exercise. This enhancement is caused by the increase of NO release, associated to K^+ channels-induced hyperpolarization (Ghafouri et al., 2011; Rossi et al., 2005; Yang et al., 2006, 2010). Additionally, Aughey et al. (2007) showed that aerobic exercise can change the activity and expression of skeletal muscle Na^+/K^+-ATPase in humans. In vascular smooth muscle, Garland et al. (2011), Marín and Redondo (1999), and Smith et al. (1997) demonstrated that Na^+/K^+-ATPase activity may be influenced by the endothelium and K^+ channels. However, there are no data in the literature showing the effect of resistance exercise on the insulin-induced relaxation nor the pathways involved in this response.

Previous research has shown the ability of resistance exercise to promote changes in vascular function in rats (Faria Tde et al., 2010; Harris et al., 2010). Interestingly, these changes can be produced in blood vessel far from the skeletal muscle used during the exercise, such as mesenteric or caudal vascular beds (Araújo et al., 2013; Faria Tde et al., 2010). Moreover, it is related in the literature that results obtained in mesenteric vascular bed may have physiological relevance

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ANEXO B – Declaração de aceite para publicação no periódico *Arquivos Brasileiros de Cardiologia* "Exercício resistido restaura a função endotelial e reduz a pressão arterial de ratos diabéticos tipo 1"

SOCIEDADE BRASILEIRA DE CARDIOLOGIA

Luiz Felipe P. Moreira
Editor-Chefe

Rio de Janeiro, 16 de dezembro de 2013.

DECLARAÇÃO

Declaro para os devidos fins, que o artigo **"Exercício Resistido Restaura a Função Endotelial e Reduz a Pressão Arterial de Ratos Diabéticos Tipo 1"**, dos autores Marcelo Mendonça Mota, Tharciano Luiz Teixeira Braga da Silva, Milene Tavares Fontes, André Sales Barreto, João Eliakim dos Santos Araújo, Antônio César Cabral de Oliveira, Rogério Brandão Wichi e Márcio Roberto Viana Santos, foi aceito para publicação no periódico Arquivos Brasileiros de Cardiologia.

Luiz Felipe P. Moreira
Editor-Chefe

ANEXO C – Declaração de aprovação do projeto de pesquisa pelo Comitê de Ética em Pesquisa com Animais da UFS

UNIVERSIDADE FEDERAL DE SERGIPE
PRÓ-REITORIA DE PÓS-GRADUAÇÃO E PESQUISA
COORDENAÇÃO DE PESQUISA
COMITÊ DE ÉTICA EM PESQUISA COM ANIMAIS (CEPA)

DECLARAÇÃO

Declaro, para os devidos fins, que o Projeto de Pesquisa intitulado "Efeitos do treinamento físico resistido na neuropatia autonômica cardiovascular diabética", sob coordenação do Prof. Dr. Márcio Roberto Viana Santos(protocolo CEPA 87/2010), foi aprovado pelo Comitê de Ética em Pesquisa com Animais da Universidade Federal de Sergipe, em reunião realizada dia 15/02/2011.

São Cristóvão, 15 de fevereiro de 2011.

Profª. Drª. Flavia Teixeira Silva
Presidente do CEPA/UFS

Cidade Universitária "Prof. Aloísio de Campos"
Jardim Rosa Elze – São Cristóvão – SE
49100-000
Fones: 3212 6661/6606

ANEXO D – Aprovação do adendo do projeto de pesquisa 47/2013 pelo Comitê de Ética em Pesquisa com Animais da UFS

UNIVERSIDADE FEDERAL DE SERGIPE
PRÓ-REITORIA DE PÓS-GRADUAÇÃO E PESQUISA
COORDENAÇÃO DE PESQUISA
COMITÊ DE ÉTICA EM PESQUISA COM ANIMAIS (CEPA)

DECLARAÇÃO

Declaro, para os devidos fins, que o Projeto de Pesquisa intitulado **"EFEITOS DO EXERCÍCIO RESISTIDO NA DISFUNÇÃO AUTOMÔMICA CARDIOVASCULAR DIABÉTICA E HIPERTENSIVA." Sob Coordenação do Prof.Dr. Márcio Roberto Viana dos Santos** (protocolo **CEPA 47/2013**) foi aprovado pelo Comitê de Ética em Pesquisa com Animais da Universidade Federal de Sergipe, em reunião realizada dia 28/02/2014.

São Cristóvão, 06 de março de 2014.

Profª. Drª. FLÁVIA TEIXEIRA SILVA
Coordenadora do CEPA/UFS

Cidade Universitária "Prof. Aloísio de Campos"
Jardim Rosa Elze – São Cristóvão – SE
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ANEXO E – Normas para publicação de artigos da *Clinical and Experimental Pharmacological and Physiological*

CEPP Clinical and Experimental Pharmacology and Physiology

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All manuscripts should be written so that they are intelligible to the professional reader who is not a specialist in the particular field. They should be written in a clear, concise, direct style. Where contributions are judged as acceptable for publication on the basis of content, the Editor and the Publisher reserve the right to modify manuscripts to eliminate ambiguity and repetition and improve communication between author and reader. If extensive alterations are required, the manuscript will be returned to the author for revision.

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Investigations in human subjects must conform to the International Ethical Guidelines for Biomedical Research Involving Human Subjects (CIOMS/WHO: http://www.cioms.ch/). Consent must be obtained from each patient or subject after full explanation of the purpose, nature and risks of all procedures used, and the fact that such consent has been given should be recorded in the paper. Patient anonymity should be observed.
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Registration in a public trials registry at or before the onset of patient enrolment is required. This policy applies to any clinical trial starting enrollment after January 1, 2006. For trials that began enrollment before this date, we request registration by April 1, 2006. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as studies on pharmacokinetics or major toxicity (e.g. phase 1 trials), are exempt. We do not advocate one particular registry. The registry should meet the following
minimum criteria: (1) accessible to the public at no charge; (2) searchable by standard, electronic (Internet-based) methods; (3) open to all prospective registrants free of charge or at minimal cost; (4) validates registered information; (5) identifies trials with a unique number; and (6) includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of last follow up, target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

Registries that currently meet these criteria include: (1) the registry sponsored by the United States National Library of Medicine (www.clinicaltrials.gov); (2) the International Standard Randomized Controlled Trial Number Registry (http://www.controlled-trials.com); (3) the Australian Clinical Trials Registry (http://www.actr.org.au); (4) the Chinese Clinical Trials Register (http://www.chictr.org); and (5) the Clinical Trials Registry - India (http://www.ctri.in); (6) University hospital Medical Information Network (UMIN) (http://www.umin.ac.jp/ctr/).

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Manuscripts should follow the style of the Vancouver agreement detailed in the *Uniform Requirements for Manuscripts Submitted to Biomedical Journals*, as presented in *JAMA* 1997; **277:** 927-34.

References are in Vancouver style. For further details regarding reference style, please see Section 10.

Spelling The Journal uses UK spelling and authors should follow the latest edition of the *Oxford Concise English Dictionary*.

Footnotes: Footnotes arising from the text must not be used.

Standard abbreviations: These should be used sparingly. They should be defined in the Summary and on the first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader.

Units of measurement: As far as possible, these should conform to the SI conventions, with the notable exception that blood pressures should be given in mmHg.
It is strongly recommended that contributors consult the booklet *Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors* (Ed. D. N. Baron), 1988, The Royal Society of Medicine Services, London. The recommendations outlined therein will be followed.

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methods used should be identified, with appropriate citation of statistical texts or articles that describe their use. Reasons for choosing particular methods of analysis, and for the number of experimental units, should be stated. The name and source of commercial statistical computer packages used should be identified. The statistical section should contain sufficient detail for the reader to have a clear idea about how the analysis was performed. The level of statistical significance (alpha) should be defined and it should be stated explicitly whether this refers to one- or two-sided probability. Nevertheless, exact *P* values should be given to a sensible number of significant figures (e.g. *P* = 0.01 rather than *P* = 0.0058). The risk of type 1 error (a false positive inference) should always be controlled. This requires the use of global statistical tests of significance where possible, particularly in experimental designs that involve repeated measurements in the same experimental units (humans, animals, tissues or cells). Multiple comparisons within a single experiment should only be made when this is absolutely necessary and, if so, *P* values should be conservatively corrected to control the risk of type 1 error. Two techniques that provide excellent control of the type 1 error rate are the 'false discovery rate' procedure (Curran-Everett D, Benos DJ. Guidelines for reporting statistics in journals published by the American Physiological Society. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. 2004; 287: R247-9) and the Ryan-Holm stepdown Bonferroni procedure (Ludbrook J. Multiple comparison procedures updated. *Clin. Exp. Pharmacol. Physiol.* 1998; 25: 1032-7). Confidence intervals may be used in addition to *P* values.

Data should be expressed as mean (SD) when the intention is to indicate the degree of variability of the data around the mean value, and as mean ± SEM when the intention is to estimate the population mean from the sample data. In either case, the numbers of observations (*n*) should be stipulated. This allows the reader to calculate SD from SEM and vice versa. (For further reading, see Ludbrook J. Comments on journal guidelines for reporting statistics. *Clin. Exp. Pharmacol. Physiol*. 2005; 32: 324-6 (Letter).)

Detailed guidelines on the use and presentation of statistics in *Clin. Exp. Pharmacol. Physiol*. can be found online at: http://otis.wiley.com/otis/journal/overview/en/5446/CEPP_Guidelines_for_the_Use_and_Presentation_of_Statistics.pdf

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2. Dale MM, Forman JC, Fan TP. *Textbook of Immunopharmacology*, 3rd edn. Blackwell Science, Oxford. 1993.

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