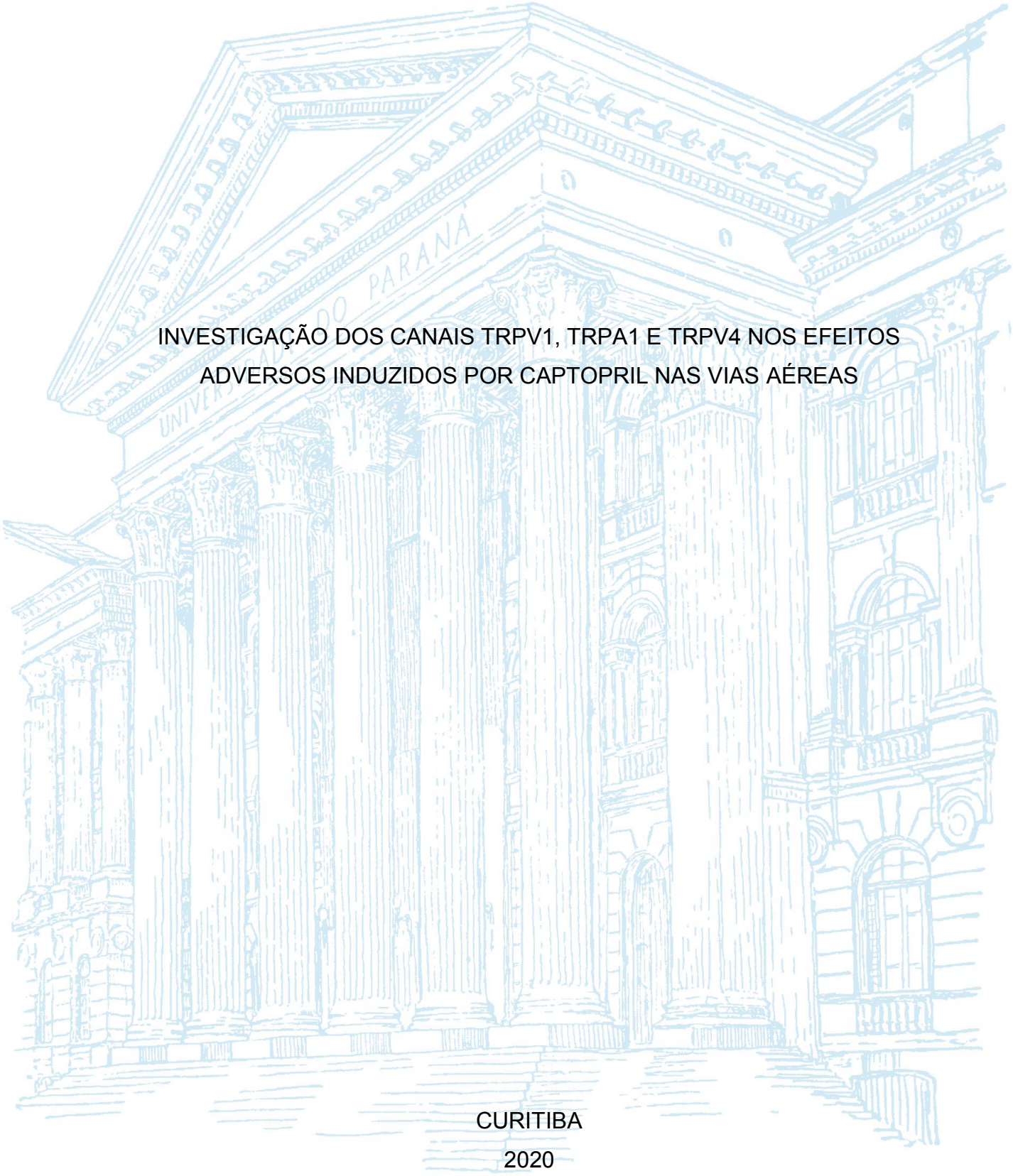
UNIVERSIDADE FEDERAL DO PARANÁ

JANIANA RAÍZA JENTSCH MATIAS DE OLIVEIRA



INVESTIGAÇÃO DOS CANAIS TRPV1, TRPA1 E TRPV4 NOS EFEITOS ADVERSOS INDUZIDOS POR CAPTOPRIL NAS VIAS AÉREAS

CURITIBA

2020

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INVESTIGAÇÃO DOS CANAIS TRPV1, TRPA1 E TRPV4 NOS EFEITOS ADVERSOS INDUZIDOS POR CAPTOPRIL NAS VIAS AÉREAS

Tese apresentada ao curso de Pós-Graduação em Farmacologia, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Farmacologia.

Orientadora: Profa. Dra. Eunice André

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em FARMACOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **JANIANA RAIZA JENTSCH MATIAS DE OLIVEIRA** intitulada: **INVESTIGACAO DOS CANAIS TRPV1, TRPA1 E TRPV4 NOS EFEITOS ADVERSOS INDUZIDOS POR CAPTOPRIL NAS VIAS AEREAS**, sob orientação da Profa. Dra. EUNICE ANDRÉ, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 30 de Outubro de 2020.

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Presidente da Banca Examinadora

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ATA Nº292

ATA DE SESSÃO PÚBLICA DE DEFESA DE DOUTORADO PARA A OBTENÇÃO DO GRAU DE DOUTOR EM FARMACOLOGIA

No dia trinta de outubro de dois mil e vinte às 09:00 horas, na sala Plataforma Jitsi meet, ambiente virtual, foram instaladas as atividades pertinentes ao rito de defesa de tese da doutoranda **JANIANA RAIZA JENTSCH MATIAS DE OLIVEIRA**, intitulada: **INVESTIGACAO DOS CANAIS TRPV1, TRPA1 E TRPV4 NOS EFEITOS ADVERSOS INDUZIDOS POR CAPTOPRIL NAS VIAS AEREAS**, sob orientação da Profa. Dra. EUNICE ANDRÉ. A Banca Examinadora, designada pelo Colegiado do Programa de Pós-Graduação em FARMACOLOGIA da Universidade Federal do Paraná, foi constituída pelos seguintes Membros: EUNICE ANDRÉ (UNIVERSIDADE FEDERAL DO PARANÁ), JOICE MARIA DA CUNHA (UNIVERSIDADE FEDERAL DO PARANÁ), SARA MARCHESAN DE OLIVEIRA (UNIVERSIDADE FEDERAL DE SANTA MARIA), ELIZABETH SOARES FERNANDES (INSTITUTO PELÉ PEQUENO PRINCIPE). A presidência iniciou os ritos definidos pelo Colegiado do Programa e, após exarados os pareceres dos membros do comitê examinador e da respectiva contra argumentação, ocorreu a leitura do parecer final da banca examinadora, que decidiu pela APROVAÇÃO. Este resultado deverá ser homologado pelo Colegiado do programa, mediante o atendimento de todas as indicações e correções solicitadas pela banca dentro dos prazos regimentais definidos pelo programa. A outorga de título de doutor está condicionada ao atendimento de todos os requisitos e prazos determinados no regimento do Programa de Pós-Graduação. Nada mais havendo a tratar a presidência deu por encerrada a sessão, da qual eu, EUNICE ANDRÉ, lavrei a presente ata, que vai assinada por mim e pelos demais membros da Comissão Examinadora.

NOTA EXPLICATIVA

Esta tese é apresentada em formato alternativo – artigos publicados e submetidos para publicação – de acordo com as normas do Programa de Pós-graduação em Farmacologia da Universidade Federal do Paraná, constando de uma revisão de literatura, objetivos do trabalho e dois artigos científicos abordando os experimentos realizados, com resultados e discussão, além da conclusão final.

Honro o fechamento deste ciclo dedicando esta tese a minha querida mãe.

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“Mas é preciso ter manha

É preciso ter graça

É preciso ter sonho sempre

Quem traz na pele essa marca

Possui a estranha mania

De ter fé na vida”

Fernando Brant / Milton Nascimento

RESUMO

Os receptores de potencial transitório (TRPs) são membros de uma família de canais iônicos conhecidos por serem expressos em neurônios sensoriais das vias aéreas e em células não neuronais do pulmão (musculatura lisa, macrófagos e células epiteliais). Embora tenha sido relatado que os TRPs apresentam um papel na modulação da função dos reflexos protetores das vias aéreas, bem como na patofisiologia das doenças pulmonares, o envolvimento desses canais nos efeitos adversos manifestados após o tratamento com inibidores da enzima conversora de angiotensina (IECAs) permanece inexplorado. O objetivo desta tese foi, portanto, determinar o papel dos canais TRPs do tipo vaniloide 1 (TRPV1), anquirina 1 (TRPA1) e vaniloide 4 (TRPV4) na hiperresponsividade, extravasamento plasmático e inflamação pulmonar induzidos pela administração de captopril, um IECA, por diferentes vias de administração e regimes de tratamento. Utilizando um modelo para avaliação da resistência das vias aéreas *in vivo* demonstramos que a administração de captopril sensibiliza os canais TRPV1, TRPA1 e TRPV4 de modo agudo, induzindo respostas brônquicas exacerbadas quando ratos Wistar machos foram desafiados com doses baixas de seus respectivos ligantes. Essa sensibilização parece depender diretamente dos níveis de bradicinina circulantes, via ativação do receptor de bradicinina tipo 2 (B_2), que, por sua vez, ativa neurônios sensoriais sensíveis à capsaicina onde os TRPs estão co-expressos. No modelo de extravasamento de plasma, demonstramos que o tratamento com captopril aumentou a permeabilidade vascular das vias aéreas, e que de modo similar a hiperresponsividade, o efeito também parece decorrer da sensibilização desses canais de modo agudo. Corroborando, aumento da contagem total de leucócitos no lavado broncoalveolar (BAL) e uma hiperplasia do tecido linfoide associado ao brônquio (BALT), também foram observados após os diferentes regimes de tratamento com captopril. E ainda, a administração sub-crônica do IECA induziu aumento do processo inflamatório e do padrão imuno-histoquímico do canal TRPV1 no pulmão de ratos. Finalmente, a degeneração de neurônios sensoriais pelo pré-tratamento neonatal com capsaicina reduziu essas respostas inflamatórias, demonstrando forte envolvimento neurogênico, embora vias não neuronais também possam participar. Em conjunto, os dados desta tese sugerem que a administração de captopril modula o limiar de ativação dos canais TRPV1, TRPA1 e TRPV4, via ativação do receptor B_2 através do aumento dos níveis circulantes de bradicinina, desencadeando respostas inflamatórias. Esse novo mecanismo sugere possibilidades para novas terapias antitussígenas e manejo farmacológico de pacientes que manifestam efeitos adversos sobre as vias aéreas durante o tratamento com IECAs, uma necessidade médica ainda não atendida.

Palavras-chave: Captopril. TRPV1. TRPA1. TRPV4. Bradicinina.

ABSTRACT

Transient potential receptors (TRPs) are members of a family of ion channels known to be expressed in sensory neurons in the airways and in non-neuronal cells of the lung (smooth muscle, macrophages and epithelial cells). Although it has been reported that TRPs play a role in modulating the function of protective airway reflexes, as well as in the pathophysiology of lung diseases, the involvement of these channels in the adverse effects manifested after treatment with angiotensin-converting enzyme inhibitors (ACEIs) remains unexplored. The objective of this thesis was, therefore, to determine the role of TRP channels type vaniloid 1 (TRPV1), anquirin 1 (TRPA1) and vaniloid 4 (TRPV4) in the hyperresponsiveness, plasma extravasation and pulmonary inflammation induced by the administration of captopril, an ACEI, in different routes of administration and treatment regimens. Using an animal model to assess airway resistance *in vivo*, we demonstrate that captopril administration sensitizes the TRPV1, TRPA1 and TRPV4 channels acutely inducing exacerbated bronchial responses when male Wistar rats were challenged with low doses of their respective ligands. This sensitization seems to depend directly on circulating bradykinin levels, via activation of the type 2 bradykinin receptor (B_2), which, in turn, activates capsaicin-sensitive sensory neurons where TRPs are co-expressed. In the plasma extravasation model, we demonstrated that treatment with captopril altered the vascular permeability of the airways, and that similarly to hyperresponsiveness, the effect also appears to result from the sensitization of these channels in an acute manner. Corroborating, an increase in the total leukocyte count in bronchoalveolar lavage (BAL) and a hyperplasia of the lymphoid tissue associated with the bronchus (BALT), were also observed after the different regimens of treatment with captopril. Furthermore, the subchronic administration of the ACEI induced an increase in the inflammatory process and alteration of the immunohistochemical pattern of the TRPV1 channel in rat lung. Finally, the degeneration of sensory neurons by the neonatal pre-treatment with capsaicin reduced these inflammatory responses, demonstrating a strong neurogenic involvement, although non-neuronal pathways may also participate. Taken together, data from this thesis suggest that the administration of captopril modulates the activation threshold of the TRPV1, TRPA1 and TRPV4 channels, via activation of B_2 receptor by increasing the circulating levels of bradykinin, triggering inflammatory responses. This new mechanism suggest possibilities for new antitussive therapies and pharmacological management of patients who manifest adverse effects on the airways during treatment with ACEIs, a medical need that has not yet been met.

Keywords: Captopril. TRPV1. TRPA1. TRPV4. Bradykinin.

LISTA DE FIGURAS

LISTA DE GRÁFICOS

MANUSCRITO ORIGINAL 1

MANUSCRITO ORIGINAL 2

LISTA DE ABREVIATURAS OU SIGLAS

4-HNE	- 4-hidroxinonenal
4α-PDD	- Didecanoato de 4α-forbol
AA	- Ácido araquidônico
AMPc	- Adenosina 3',5'-monofosfato cíclico
ANG II	- Angiotensina II
ANOVA	- Análise de variância
AT1	- Receptor de angiotensina II tipo 1
ATP	- Adenosina trifosfato
B_1	- Receptor de bradicinina tipo 1
B_2	- Receptor de bradicinina tipo 2
BAL	- Lavado bronco alveolar
BALT	- Tecido linfoide associado ao brônquio
CaMKII	- Proteína quinase dependente de cálcio/calmodulina
CAP	- Captopril
CAPES	- Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CIENP	- Centro de Inovação e Ensaios Pré-Clínicos
CNPQ	- Conselho Nacional de Desenvolvimento Científico e Tecnológico
CPZ	- Capsazepina
CGRP	- Peptídeo relacionado ao gene da calcitonina
DABK	- Des-arg^9-bradicinina
DAG	- Diacilglicerol
DEG	- Grupo de animais pré-tratados com capsaicina no período neonatal
DMSO	- Dimetilsulfóxido
DRG	- Gânglio da raiz dorsal
DPOC	- Doença pulmonar obstrutiva crônica
ECA	- Enzima conversora de angiotensina
et al.	- E outros
EUA	- Estados Unidos da América
IECAs	- Inibidores da enzima conversora de angiotensina
H&E	- Hematoxilina e eosina
I.P.	- Via intraperitoneal
I.T.	- Via intratraqueal

I.V. - Via intravenosa
FIG - Figura
FINEP - Financiadora de Estudos e Projetos
GPCR - Receptores acoplados à proteína G
IP3 - Inositol (1,4,5)-trifosfato
NAPQI - N-acetil-p-benzo-quinona imina
NGF - Fator de crescimento nervoso
OTC - Medicamentos isentos de prescrição médica
P2X3 - Receptores purinérgicos família P2X subunidade 3
PBS - Tampão fosfato-salino
PGE2 - Prostaglandina E2
PIP2 - Fosfatidilinositol (4,5)-bifosfato
PKA - Proteína quinase A
PKC - Proteína quinase C
PLC - Fosfolipase C
RA - Resistência das vias aéreas
RARs - Receptores de adaptação rápida
ROS - Espécies reativas de oxigênio
RTKs - Receptores tirosina quinases
SARs - Receptores de adaptação lenta
SNC - Sistema nervoso central
SRAA - Sistema renina-angiotensina-aldosterona
TRPs - Receptores de potencial transitório
TRPM - Receptor de potencial transitório melastatina
TRPA - Receptor de potencial transitório anquirina
TRPA1 - Receptor de potencial transitório do tipo anquirina 1
TRPC - Receptor de potencial transitório canônico
TRPML - Receptor de potencial transitório mucolipina
TRPP - Receptor de potencial transitório policistina
TRPV - Receptor de potencial transitório vanilóide
TRPV1 - Receptor de potencial transitório do tipo vaniloide 1
TRPV4 - Receptor de potencial transitório do tipo vaniloide 4
VEI - Veículo
V.O. - Via oral

LISTA DE SÍMBOLOS

ºC	- Grau Celsius
α	- Alfa
β	- Beta
±	- Mais ou menos
>	- Maior
<	- Menor
≤	- Menor ou igual
%	- Por cento
Ca^{2+}	- Cálcio
Mg^{2+}	- Magnésio
δ	- Delta
µg	- Micrograma
µl	- Microlitro
µmol/kg	- Micromol por quilograma
g	- Grandeza de aceleração da centrífuga
mg	- Miligrama
mg/kg	- Miligrama por quilograma
m/s	- Metros por segundo
NaCl	- Cloreto de sódio
kg	- Quilograma
pH	- Potencial hidrogeniônico
s	- Segundos
x	- lê-se a (“contra”)

SUMÁRIO

1 INTRODUÇÃO

1.1 INIBIDORES DA ENZIMA CONVERSORA DE ANGIOTENSINA

Inibidores da enzima conversora de angiotensina (IECAs) estão no mercado a mais de 30 anos e se estabeleceram como uma das maiores terapias cardiovasculares. Após Ferreira e colaboradores demonstrarem em 1970 que uma fração do veneno da *Bothrops jararaca* era capaz de potencializar as ações da bradicinina *in vivo* e *in vitro* por inibir a enzima conversora de angiotensina (ECA) (FERREIRA et al., 1970), Ondetti e Cushmann sintetizaram o primeiro IECA para administração oral, o fármaco captopril, em 1981 (ONDETTI; CUSHMAN, 1981).

O captopril, IECA de primeira geração, formou a base para os compostos subsequentes e atualmente mais de dez IECAs estão disponíveis para uso clínico e se diferenciam em 3 classes de acordo com o seu grupo funcional; grupamento sulfidrila (captopril e zofenopril), dicarboxilato (enalapril, lisinopril, ramipril, entre outros) e fosfonato (fosinopril), o que confere a eles diferenças no seu perfil farmacodinâmico e cinético (TADDEI; BORTOLOTTO, 2016; LAURENT, 2017).

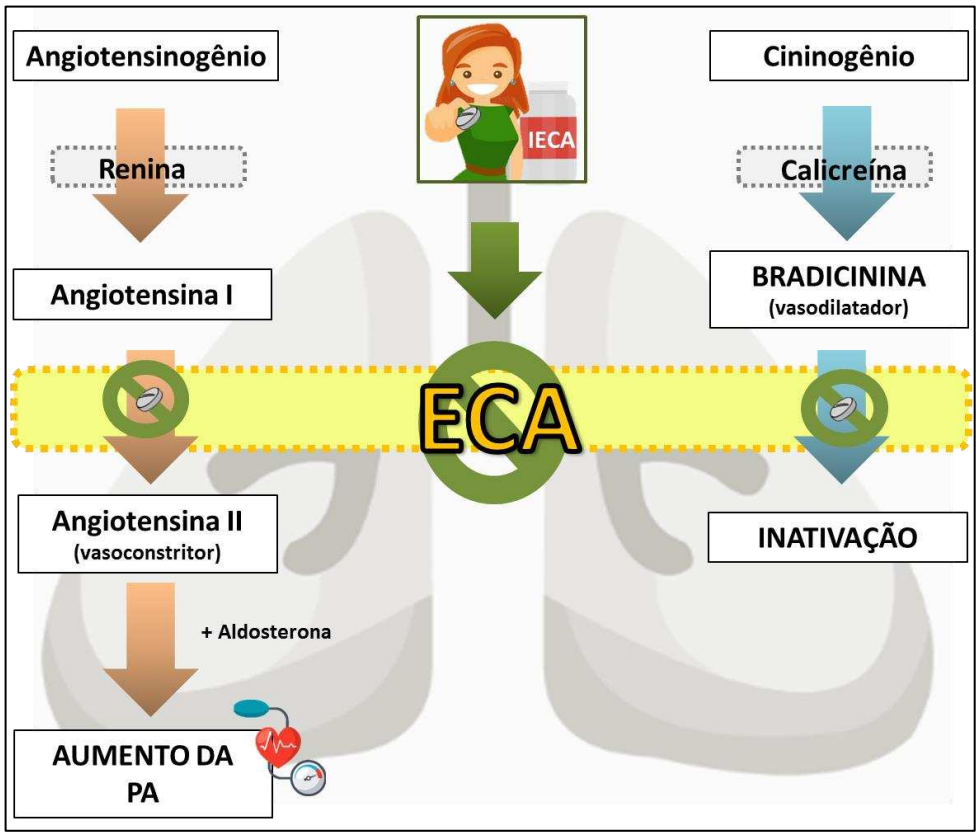
Essa classe de medicamentos é considerada como tratamento de primeira linha para o gerenciamento da hipertensão sistólica/diastólica em adultos segundo os guidelines canadense (NERENBERG et al., 2018), americano (WHELTON et al., 2018), europeu (WILLIAMS et al, 2018) e brasileiro (MALACHIAS et al., 2016) recentes. Além disso, possuem indicação no tratamento da insuficiência cardíaca congestiva, infarto do miocárdio e outros eventos coronarianos, com redução de 35%, 48% e 35%, respectivamente, com base em uma meta-análise com mais de 195 mil pacientes (TADDEI; BORTOLOTTO, 2016). Adicionalmente, IECAs favorecem a diminuição da progressão da nefropatia diabética e não diabética (ACHARYA et al., 2003). Por esses motivos, estão entre os fármacos mais prescritos e comercializados globalmente.

Estima-se que 35% de todas as prescrições de medicamentos anti-hipertensivos nos Estados Unidos da América (EUA) sejam de IECAs, e que mais de 40 milhões de pessoas no mundo administrem esses medicamentos diariamente (BANERJI et al., 2016). No ano de 2015, por exemplo, estima-se que 34 bilhões de dólares foram gastos para aquisição dessas drogas no mundo, um dos cinco medicamentos mais prescritos globalmente (LIAU et al., 2019).

Seu mecanismo de ação visa reestabelecer a homeostasia da pressão arterial, da perfusão tecidual e do volume extracelular por meio da redução do desequilíbrio entre os sistemas renina-angiotensina-aldosterona (SRAA) e o sistema calicreínas-cininas (REGOLI; GOBEIL, 2015). Esses sistemas estão interligados pela ECA, uma dipeptidilcarboxidase localizada nas células endoteliais de vasos, capilares, vênulas e células endoteliais pulmonares. A ECA é responsável por catalisar a conversão de angiotensina I em angiotensina II (ANG II), componente chave do SRAA que, mediante ativação de seus receptores metabotrópicos do tipo 1 (AT1), estimula a síntese e liberação de aldosterona, a retenção de íons sódio e a vasoconstrição (ZAMAN; OPARIL; CALHOUN, 2002; ACHARYA et al., 2003; LAURENT, 2017). Adicionalmente, 75% a 95% da bradicinina, agente vasodilatador componente chave do sistema calicreína-cininas, que se opõe aos efeitos da ativação do SRAA, é metabolizada pela mesma enzima a metabólitos inativos (REGOLI; PLANTE; GOBEIL, 2012). Por esse motivo, a ECA algumas vezes é referida como cininase II (ACHARYA et al., 2003).

Quando IECAs se ligam competitivamente ao sítio ativo da enzima, eles impedem a clivagem dos seus substratos, angiotensina 1 e bradicinina, reduzindo a atividade do SRAA e potencializando as ações do sistema calicreína-cininas, respectivamente (BICKET, 2002). E então, no geral, IECAs são efetivos em uma alta porcentagem de pacientes, geralmente bem tolerados e considerados relativamente seguros (FIGURA 1).

FIGURA 1 – REPRESENTAÇÃO ESQUEMÁTICA DA AÇÃO FARMACOLÓGICA DE INIBIDORES DA ENZIMA CONVERSORA DE ANGIOTENSINA



A ECA, também conhecida como cininase II, é expressa no endotélio vascular dos principais órgãos-alvo, incluindo pulmão. Esta enzima catalisa a conversão do decapeptídeo inativo angiotensina I ao octapeptídeo biologicamente ativo angiotensina II e a hidrólise da bradicinina a produtos inativos, regulando a função cardiovascular, pressão arterial e renal na saúde e na doença. Logo, quando IECAs se ligam ao sítio ativo da enzima, interferem com a sua habilidade em clivar seus substratos, diminuindo as ações do eixo clássico angiotensinogênio/renina/ECA/ANG II/AT1 e potencializam as ações do sistema cininas/caclicreínas/bradicinina (ZAMAN; OPARIL; CALHOUN, 2002; LI; ZHANG; ZHUO, 2017).

1.2 EFEITOS ADVERSOS E PREVALÊNCIA

Efeitos adversos não passíveis de previsão são razões comuns para baixa adesão à terapia com IECAs (ISRAILI; HALL, 1992; VASEKAR; CRAIG, 2012). O efeito adverso mais comum (5 a 25% de incidência) em pacientes em tratamento é a tosse seca, seguida por hipotensão (4,1%), angioedema (1,3%) e disfunção renal (1%) (SESOKO; KANEKO, 1985; BAS et al., 2010; ACHARYA et al., 2003; MORIMOTO et al., 2004; OMBONI; BORGHI, 2011). Rash cutâneo, perda de sabor dos alimentos, aumento da reatividade brônquica e broncoespasmo, infiltrados

pulmonares intersticiais, neutropenia e aumento do risco de câncer de pulmão também foram descritos na literatura (BUCKNALL, 1988; OBERGASSEL; CARLSSON; TEBBE, 1996; OVERLACK, 1996; HICKS et al., 2018; YILMAZ, 2019).

Uma maior incidência desses efeitos foi observada em pacientes com descendência africana (acredita-se que tenham sensibilidade aumentada para bradicinina), pessoas do sexo feminino e fumantes (WAKEFIELD; THEAKER; PEMBERTON, 2008; BONNER et al., 2017). Até o momento, não há relato de uma associação temporal entre o início do tratamento com IECAs e a ocorrência dos efeitos adversos, assim como não há evidência de uma relação dose-resposta (WAKEFIELD; THEAKER; PEMBERTON, 2008). Logo, os efeitos são imprevisíveis e podem se manifestar nas primeiras horas após a primeira dose padrão ou semanas a meses após o início da terapia (DICPINIGAITIS, 2006). Qualquer IECA pode causar esses efeitos, embora a maior parte dos estudos descrevam reações após o tratamento com captopril e enalapril (O'RYAN; POOR; HATTORI, 2005).

1.2.1 Tosse e hipersensibilidade

A tosse representa um dos mais potentes mecanismos para proteção das vias aéreas. Em condições normais, a tosse é um reflexo defensivo vital, dirigida por fibras sensoriais nervosas responsáveis por detectar alterações no ambiente físico e químico. Contempla as fases de inspiração, compressão e expiração, para que um fluxo de ar adequado seja gerado de modo a expelir materiais estranhos, partículas e muco das paredes das vias aéreas (BROUNS et al., 2012). No entanto, em situações patofisiológicas específicas que podem ocorrer durante a doença, a tosse pode se tornar excessiva e uma condição crônica, o que pode ser prejudicial à mucosa das vias aéreas e impactar negativamente na qualidade de vida dos pacientes (distúrbios do sono, dores no peito, náuseas e letargia) (GEPPETTI et al, 2010; CANNING et al., 2014). Além disso, existe um custo econômico significativo para o indivíduo com tosse e para sociedade, uma vez que pode levar à ausência do trabalho e à diminuição da produtividade (DICPINIGAITIS, 2014).

A incidência de tosse induzida por IECAs varia de acordo com a etnia do paciente, sendo alta em populações asiáticas (45%) e menor em caucasianos (10%) (LIAU et al., 2019). Muitos pacientes com tosse crônica apresentam a síndrome da hipersensibilidade à tosse, caracterizada pela diminuição do limiar a estímulos

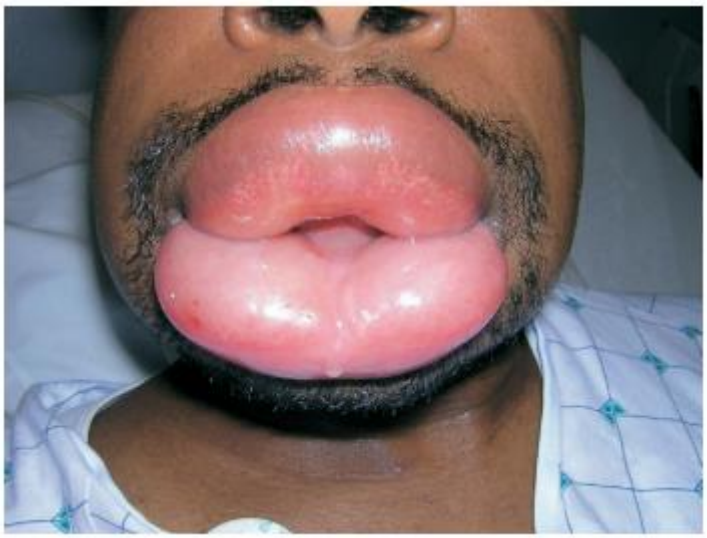
nocivos (fumaça do cigarro, por exemplo) e alterações na temperatura (CHUNG, 2011; FARUQI et al., 2014). Os sintomas incluem além da tosse, dificuldade para respirar, rouquidão, rinorreia e irritação ocular (MILLQVIST, 2011). Frequentemente, pacientes tratados com IECAs apresentam maior sensibilidade ao reflexo da tosse (SONG; MORICE, 2017). Morice e colaboradores demonstraram que a concentração de capsaicina inalada (irritante agonista do receptor de potencial transitório do tipo vaniloide 1, TRPV1) necessária para induzir tosse foi menor em indivíduos saudáveis que receberam a administração de 25 mg de captopril (MORICE et al., 1987).

Apesar de não estar totalmente elucidado, o principal mecanismo da hipersensibilidade à tosse tem sido sugerido como vias neurais sensoriais desreguladas, uma vez que a regulação positiva funcional dos nervos sensoriais epiteliais das vias aéreas é comumente encontrada em pacientes que sofrem com tosse crônica (GRONEBERG et al., 2004). Assim, em resumo, este efeito é descrito como uma hipersensibilidade das vias aéreas, onde a hiperreatividade dos neurônios sensoriais promove maior resposta da tosse à capsaicina inalada (MILLQVIST, 2011). Logo, a administração de IECAs poderia sensibilizar o reflexo da tosse, potencializando outras causas de tosse crônica nos pacientes (DICPINIGAITIS, 2006).

1.2.2 Angioedema

Angioedema é uma condição clínica caracterizada por inchaço dos tecidos subcutâneos e submucosos devido ao extravasamento vascular. O angioedema induzido por IECAs acomete a face, lábios, língua, pescoço e o trato superior das vias aéreas, sem a presença de urticaria ou desenvolvimento de anticorpos, levando à rouquidão, incapacidade de deglutição e dificuldade em respirar (GRANT; DEEB; CHIA, 2007; JEON; LEE; LEE, 2019) (FIGURA 2).

FIGURA 2 – FOTOGRAFIA DE UM PACIENTE ACOMETIDO POR UM ANGIOEDEMA, LIMITADO AOS LÁBIOS, DECORRENTE DA ADMINISTRAÇÃO DE UM IECA



Fonte: GRANT; DEEB; CHIA, 2007.

Apesar de relativamente incomum, o efeito está associado a uma considerável morbidade e pode ser fatal. Com frequência pacientes com edema de língua e laringe por angioedema causados por IECAs exigem admissão na unidade de tratamento intensivo para intervenção nas vias aéreas, permanecendo intubados por 24 a 72 horas até a alta hospitalar (O'RYAN; POOR; HATTORI, 2005). Sua prevalência é cinco vezes maior na população africana americana do que em caucasianos. Além disso, ser do sexo feminino, possuir idade acima de 65 anos, ter o hábito de fumar, ter se submetido à cirurgia das vias aéreas superiores, apresentar histórico de asma e alergias, são fatores de risco para o seu desenvolvimento (CAMPO et al., 2013).

Infelizmente até o momento não existe teste laboratorial definitivo para diagnosticar este efeito (BAS; STORCK; STRASSEN, 2017). Sendo assim, a descontinuação do tratamento com o IECA e a monitorização até a resolução dos sintomas são as ferramentas clínicas disponíveis para sua confirmação (VASEKAR; CRAIG, 2012).

1.3 TERAPIAS DISPONÍVEIS PARA AMENIZAR OS EFEITOS ADVERSOS INDUZIDOS POR IECAS NAS VIAS AÉREAS

Até o momento não há uma intervenção farmacológica definitiva e aprovada para o tratamento de angioedema, tosse ou inflamação pulmonar induzido por IECAs, uma vez que seus mecanismos patofisiológicos não foram totalmente elucidados. O manejo recomendado consiste em suspender a administração do fármaco e substituí-lo por uma classe alternativa, visto que a troca por outro IECA pode fazer com que os efeitos adversos recorram (OVERLACK, 1996).

A interrupção da administração do fármaco é único tratamento uniformemente eficaz e faz com que os sinais e sintomas de angioedema geralmente se resolvam entre 24 – 48 horas (VASEKAR; CRAIG, 2012), enquanto a tosse geralmente se resolve dentro de 1 a 4 semanas após a descontinuação da terapia (DICPINIGAITIS, 2006). No entanto, quando da suspensão do medicamento, os pacientes ficam expostos ao risco de sua situação atual, como crises hipertensivas. Logo, a prevenção e resolução dos efeitos adversos induzidos por IECAs nas vias aéreas de pacientes são limitadas.

A prescrição de antitussígenos para tosse induzida por IECAs ao invés da descontinuação e substituição do fármaco constitui uma farmacoterapia irracional, resultando muitas vezes em polifarmácia e exposição aos efeitos adversos dos antitussígenos (VEGTER; DE JONG-VAN DEN BERG, 2010). Medicamentos isentos de prescrição médica mostram pouca eficácia no alívio da tosse induzida por IECAs e os opiáceos, padrão ouro no tratamento da tosse, estão associados a efeitos colaterais moderados a graves, como sedação, náusea e dependência física (DICPINIGAITIS, 2006).

Em se tratando do angioedema induzido por IECAs, situação em que, ao contrário da tosse seca, há risco de morte por asfixia, a busca por um tratamento eficaz é emergente. Especialmente quando ocorre comprometimento das vias aéreas superiores, medicamentos como anti-histamínicos, anticolinérgicos, corticosteroides e adrenalina são frequentemente utilizados. No entanto, apresentam nenhum ou efeito limitado (NIELSEN; BYGUM; RASMUSSEN, 2016). Assim, devido à dificuldade de um tratamento aprovado, o cuidado de suporte, associado a uma maior morbidade, e que inclui procedimentos invasivos como intubação,

traqueotomia e cricotireoidetomia, com maior tempo de hospitalização e admissão na unidade de terapia intensiva, podem ser necessários (BONNER et al., 2017).

O medicamento ecalantide, por exemplo, um inibidor de calicreína com eficácia comprovada no angioedema hereditário foi estudado, porém, não foi efetivo no tratamento de angioedema induzido por IECAs (NIELSEN; BYGUM; RASMUSSEN, 2016). Outra possibilidade, o plasma fresco congelado, demonstrou capacidade de aliviar os sintomas do angioedema por IECA embora existam chances de reações de hiperssenssibilidade (NIELSEN; BYGUM; RASMUSSEN, 2016; BERNSTEIN et al., 2017). Há também relatos na literatura de que a administração de icatibanto, potente e seletivo antagonista do receptor B_2 também chamado de HOE140, seria capaz de inibir os efeitos vasculares da bradicinina quando administrado na dose de 30 mg por via subcutânea (BAS et al., 2010; BERNSTEIN et al., 2017). No entanto, devido ao baixo número de pacientes envolvidos no estudo, os dados são conflitantes, e não foram confirmados em uma meta análise recente (BONNER et al.,2017; JEON; LEE; LEE, 2019). Nesse sentido, uma terapia farmacológica efetiva e aprovada para resolução desses efeitos adversos nas vias respiratórias é uma necessidade médica ainda não atendida. E por esse motivo, estudos para acessar a patogênese destes efeitos e determinar alvos potenciais para o seu tratamento continuam a ser realizados.

1.4 PATOGÊNESE DOS EFEITOS ADVERSOS INDUZIDOS POR IECAS NAS VIAS AÉREAS

O mecanismo dos efeitos adversos decorrentes da administração de IECAs não foi totalmente elucidado, embora tenha sido atribuído a alteração dos níveis de bradicinina. Nussberger e colaboradores demonstraram que pacientes tratados com IECA apresentam níveis plasmáticos de bradicinina significativamente aumentados (NUSSBERGER et al., 1998), assim como em pacientes durante um ataque de angioedema (BONNER et al.,2017). De fato, a inibição da ECA favorece o acúmulo desse importante mediador no pulmão, capaz de promover os principais sinais da inflamação (DICPINIGAITIS, 2006). Em ratos, essa inibição farmacológica aumenta os níveis de cininas nos vasos, rim, pulmão, coração e tecido adiposo (REGOLI; PLANTE; GOBEIL, 2012).

Os inibidores da ECA também promovem a potencialização da sinalização da bradicinina não somente por reduzir a sua degradação, mas também por atuarem como agonistas alostéricos diretos de receptores B_1 e indiretos de receptores B_2 (ERDÖS, FULONG; SKIDGEL, 2010) bem como por favorecer a inibição da dessensibilização dos receptores B_2 (ACHARYA et al., 2003). Logo, além das evidências sugerindo que a bradicinina, agindo em seus receptores específicos, desempenhe efeitos inflamatórios e um papel importante nos efeitos adversos induzidos por IECAs, outros mecanismos podem de modo direto e indireto potencializar as ações desse mediador inflamatório. Assim, o acúmulo de bradicinina é dificilmente aceito como o único mecanismo, uma vez que este mediador possui uma meia vida plasmática curta, os efeitos adversos são imprevisíveis e não dose-dependentes.

1.4.1 O papel da bradicinina

A bradicinina é um nonapeptídeo vasoativo potente que participa de processos inflamatórios por causar aumento da permeabilidade vascular, o recrutamento de células inflamatórias, a contrição da musculatura lisa uterina e gastrointestinal, broncoconstrição, ativação da fosfolipase A_2 e liberação de prostanoides, taquicininas e citocinas (KAPLAN; JOSEPH, 2014; RICCIARDOLO et al., 2018). A via clássica de produção de bradicinina no plasma humano consiste de 3 proteínas, o fator XII (fator de Hageman), a pré-calicreina e o cininogênio de alto peso molecular, e integra o sistema chamado calicreína-cininas. Essas proteínas estão ligadas a células vasculares endoteliais. Resumidamente, a ativação/perturbação de tais células promove a ativação do fator XII, seguido pela conversão da pré- calicreína em calicreína, que por sua vez digere o cininogênio de alto peso molecular liberando a bradicinina (para revisão ver KAPLAN; JOSEPH, 2014).

A regulação de suas ações envolve a sua inativação por várias peptidases como a aminopeptidase P, endopeptidase neutra e carboxipeptidases M e N e a ECA, a principal (CICARDI; ZURAW, 2018). A ECA está abundantemente expressa na superfície vascular de células endoteliais pulmonares. Logo, a degradação da bradicinina ocorre largamente na vasculatura pulmonar, com tempo de meia-vida de cerca de 17 segundos na corrente sanguínea (LUMB, 2010). Assim, o bloqueio de

sua inativação pela inibição da ECA, resulta em prolongamento do seu tempo de meia-vida e aumento de sua atividade biológica (YILMAZ, 2019).

Seus efeitos farmacológicos são mediados pela ativação dos receptores de bradicinina tipo B_1 e B_2, caracterizados por Regoli e colaboradores no final de 1970 (HALL, 1997). Ambos são membros de uma superfamília de receptores acoplados a diferentes tipos de proteína G (GPCRs) expressos na membrana plasmática, que sinalizam primariamente via proteínas Gαq/11 e Gαi/o, mas também independentemente através de efetores intracelulares (MAURER et al., 2011).

Os receptores apresentam um padrão de expressão diferente nos tecidos. Receptores B_1 são induzíveis, regulados por lesão tecidual durante processos inflamatórios, possuem baixa afinidade para a bradicinina e maior afinidade por seus metabólitos provenientes da ação da enzima cininase I (carboxipeptidase N) sobre a bradicinina (des-arg^9 -bradicinina e lys-des-arg^9–bradicinina) (CALIXTO et al., 2004; RHALEB, YANG; CARRETERO, 2011). Os receptores B_2 são constitutivos, expressos em densidade relativamente constante, e possuem alta afinidade por bradicinina (ABRAHAM; SCURI; FARMER, 2006). Estão amplamente distribuídos na vasculatura endotelial, neurônios sensoriais, células da musculatura lisa, epitélio respiratório e alguns tipos de leucócitos (MARCEAU et al., 2018). Logo, a maior parte dos efeitos farmacológicos da bradicinina sobre as vias aéreas são gerados via ativação de receptores B_2 (LEEB-LUNDBERG et al., 2005)

Resumidamente, a ativação do receptor B_2 acoplado a proteína Gαq por bradicinina estimula a enzima fosfolipase C (PLC) a hidrolisar o fosfatidilinositol (4,5)- bifosfato (PIP2), aumentando os níveis de segundo mensageiros como cálcio (Ca^{2+}), inositol (1,4,5)-trifosfato (IP3) e diacilglicerol (DAG) (MARCEAU et al., 2018). O aumento da liberação de íons Ca^{2+} dos estoques celulares, por sua vez, auxilia a liberação de neuropeptídeos por neurônios sensoriais primários e a ativação de isoformas específicas de proteína quinase C (PKC) que podem fosforilar proteínas alvo (LEEB-LUNDBERG et al., 2005; PETHÖ; REEH, 2012). Em conjunto, são geradas respostas como o extravasamento de plasma, broncoconstrição e hiperreatividade brônquica (TRAMONTANA et al., 2001; GAMA LANDGRAF et al., 2004; SULPIZIO et al., 2004; VALENTI et al., 2005), efeitos comumente associados com a patofisiologia de doenças respiratórias (FULLER et al., 1987) e que podem desempenhar papel importante nos efeitos adversos induzidos por IECAs nas vias aéreas.

Emanueli e colaboradores (1998) demonstraram que captopril induz extravasamento plasmático em camundongos via ativação de receptor B_2, visto que o efeito é inibido pelo pré-tratamento com HOE140 (icatibanto) e abolido quando da deleção genética do mesmo receptor (EMANUELI et al., 1998). Corroborando, Katsumata et al. (1991) demonstraram que pacientes tratados com IECA apresentam um aumento acentuado da tosse induzida por bradicinina inalada (KATSUMATA et al., 1991). No entanto, estudos adicionais geraram evidências de que bradicinina poderia também promover suas respostas nas vias aéreas por ativar diferentes subtipos de fibras aferentes sensoriais (CANNING, 2010). Fox e colaboradores (1996) demonstraram que a bradicinina sensibiliza fibras sensoriais do tipo C (FOX et al.,1996), onde receptores B_2 estão coexpressos com receptores de potencial transitório (TRPs) (PETHÖ; REEH, 2012; VELDHUIS et al., 2015). Essa sensibilização, por sua vez, aumentou o número de tosses provocadas pelo tratamento com IECA em cobaias. (FOX et al., 1996). O extravasamento plasmático induzido por captopril em ratos mecanicamente ventilados também parece envolver a participação dos receptores B_2 e neurônios sensoriais sensíveis a capsaicina das vias aéreas (DE OLIVEIRA et al., 2016). Nesse sentido, o conhecimento dos diferentes subtipos de nervos aferentes sensoriais vagais que controlam a respiração é fundamental para a compreensão e investigação da patofisiologia dos efeitos adversos manifestados durante o tratamento com IECAs.

1.5 NEURÔNIOS SENSORIAIS QUE CONTROLAM A RESPIRAÇÃO

As vias aéreas utilizam uma inervação aferente densa, derivada principalmente, mas não exclusivamente, de neurônios nos gânglios sensoriais vagais para detectar alterações no ambiente físico e químico local e levar essas informações ao sistema nervoso central, causando respostas reflexas (BROUNS et al., 2012). A comunicação neuronal entre o trato respiratório e o sistema nervoso central (SNC) depende dos potenciais de ação gerados nos terminais nervosos aferentes e conduzidos para os terminais centrais do SNC (MAZZONE; UNDEM, 2016). Assim, reflexos centrais e locais como tosse, broncoconstrição, secreção de muco e vazamento microvascular são mediados (NASRA; BELVISI, 2009).

Os corpos celulares dos neurônios sensoriais vagais que inervam o trato respiratório se originam em dois gânglios distintos, denominados gânglio nodoso e

gânglio jugular. Esses nervos aferentes, nodoso e jugular, têm fenótipos distintos e, portanto, provavelmente subservem funções distintas, consistindo diferentes subtipos dentro das vias aéreas (NASRA; BELVISI, 2009; GRACE et al, 2011; MAZZONE; UNDEM, 2016).

1.6 SUBTIPOS DE NEURÔNIOS SENSORIAIS DAS VIAS AÉREAS

1.6.1 Receptores de estiramento pulmonar (fibras Aβ)

As fibras aferentes vagais que terminam no trato respiratório que conduzem potenciais de ação na faixa Aβ são em grande parte caracterizados por sua respostas à inflação pulmonar sustentada. As fibras Aβ sensíveis que transmitem informação mecânica são convencionalmente subcategorizadas com base na adaptação do potencial de ação como receptores de adaptação lenta (SARs) e receptores de adaptação rápida (RARs) (MAZZONE; UNDEM, 2016). Ambos desempenham um papel crítico na regulação da freqüência respiratória e volume corrente (CANNING et al., 2014).

RARs foram nomeados refletindo sua adaptação rápida às inflações pulmonares sustentadas. Possuem seus corpos celulares no gânglio nodoso e são insensíveis à estimulação direta por estímulos químicos como bradicinina e capsaicina, embora esses estímulos possam indiretamente ativar essas fibras, gerando broncoconstrição, produção de muco ou edema (NASRA; BELVISI, 2009). SARs são altamente sensíveis às forças mecânicas impostas ao pulmão durante a respiração e geralmente insensíveis a estímulos químicos (LEE; YU, 2014). São responsáveis por ajustar o padrão de respiração, diminuir a inspiração e prolongar a expiração. Estão envolvidos no reflexo de broncodilatação, taquicardia e vasodilatação sistêmica, sem efeito direto na tosse (CANNING et al., 2014; POLVERINO et al., 2012).

1.6.2 Receptores de tosse

Estudos em cobaias demonstraram a existência de um grupo de fibras A, mielinizadas, que se originam nos gânglios nodosos, e que quando estimuladas levam à tosse, terminando quase exclusivamente nos brônquios extrapulmonares, traqueia e laringe (NASRA; BELVISI, 2009). Sua velocidade de condução é 5 vezes

mais rápida que as fibras C e cinco vezes mais lenta que as fibras Aβ RARs e SARs, indicando um perfil de fibra Aδ. São sensíveis a estimulação mecânica pontual do epitélio, mudanças rápidas do pH luminal e soluções hipotônicas, e não responsivas a estimulação química por capsaicina ou bradicinina (CANNING, 2010; GRACE et al., 2011; LEE; YU, 2014; MAZZONE; UNDEM, 2016). Curiosamente, ratos e camundongos não possuem essas fibras e apresentam o reflexo da tosse menos desenvolvido (NASRA; BELVISI, 2009).

Fibras Aδ quimicamente sensíveis, que expressam o canal TRPV1 e não sintetizam neuropeptídios, também foram descritas nas vias aéreas de algumas espécies, com responsividade a bradicinina e a capsaicina (GRACE et al., 2013; LEE; YU, 2014). No entanto, seu papel nos reflexos homeostáticos e defensivos das vias aéreas ainda é desconhecido (MAZZONE; UNDEM, 2016).

1.6.3 Aferentes quimicamente sensíveis

A maioria dos nervos aferentes que inervam as vias aéreas e exibem sensibilidade a uma gama de estímulos químicos, menos sensíveis a perturbações mecânicas, são fibras aferentes do tipo C não mielinizadas (LEE; PISARRI, 2001). Essas compreendem aproximadamente 75% dos nervos aferentes que inervam as vias aéreas, com velocidade de condução de 0,3 - 2 m/s e, a maioria, caracterizadas pela expressão do canal iônico TRPV1 (sensível a capsaicina), além da expressão de outros canais de cátions operados por ligantes e GPCRs (MAZZONE; UNDEM, 2016). Essas fibras podem ser sub-classificadas como fibras C pulmonares ou fibras C brônquicas, com base na acessibilidade circulatória, recebendo perfusão sanguínea da circulação pulmonar ou suprimento sanguíneo da circulação brônquica, respectivamente (LEE; PISARRI, 2011).

As fibras C estão geralmente inativas durante todo o ciclo respiratório, mas se ativam de modo direto por estímulos químicos como extratos alimentares (capsaicina, óleo de mostarda, wasabi e gengibre), substâncias irritantes do ambiente (fumaça de cigarro, poluição do ar e escapamentos de veículos) e mediadores endógenos (por exemplo, bradicinina, prostanóides e produtos de oxidação) (GRACE et al., 2013). Uma subpopulação de fibras C sintetiza neuropeptídeos nos seus corpos celulares nos gânglios nodoso e jugular, tais como substancia P, neurocinina A e peptideo relacionado ao gene da calcitonina (CGRP),

que são subsequentemente transportados e armazenados em seus terminais nervosos periféricos (LEE; PISARRI, 2001; MAZZONE; UNDEM, 2016). Quando essas terminações são estimuladas no trato respiratório, impulsos desencadeiam a liberação desses neuropeptídios, capazes de agir em células efetoras e gerar efeitos reflexos locais como broncoconstrição, quimiotaxia de células inflamatórias, extravasamento plasmático e edema das mucosas das vias aéreas, coletivamente referidos como inflamação neurogênica (LEE; PISARRI, 2001; NASRA; BELVISI, 2009). Adicionalmente, a estimulação de fibras C brônquicas e pulmonares pode também desencadear profundas respostas respiratórias mediadas por reflexos centrais, incluindo constrição das vias aéreas, secreção de muco, tosse e taquipneia (BROUNS et al., 2012).

1.7 TRPS, REFLEXOS DAS VIAS AÉREAS E INFLAMAÇÃO PULMONAR

Os neurônios sensoriais das vias aéreas são conhecidos por expressar uma variedade de receptores e canais iônicos, que são ativados por mediadores endógenos e exógenos, como por exemplo, a família dos TRPs. Esses canais são proteínas seletivas a cátions, que exibem uma preferência geral por íons cálcio e que desempenham um importante papel nos mecanismos protetores das vias aéreas (GRACE et al., 2014). Os TRPs foram descobertos no olho da mosca *Drosophila melanogaster* e nomeados por sua resposta transitória à luz brilhante (MONTELL; RUBIN, 1989). Exibem seis domínios transmembranares com o poro do canal localizado entre os domínios 5 e 6, terminais C e N intracelulares e graus variados de repetição de anquirina (JULIUS, 2013).

TRPs estão classificados em 6 subfamílias, com base na sua sequência de aminoácidos, em TRPV (vanilóide), TRPM (melastatina), TRPA (anquirina), TRPML (mucolipina), TRPP (policistina) e TRPC (canonico) (NILIUS; SZALLASI, 2014). As evidências atuais sugerem que os canais TRP ativos são formados por quatro subunidades e que podem se reunir como homo ou heterotetrâmeros (MICKLE; SHEPHERD; MOHAPATRA, 2015). No que tange as vias aéreas, os receptores vaniloide tipo 1 (TRPV1), vaniloide tipo 4 (TRPV4) e anquirina tipo 1 (TRPA1) apresentam maior interesse devido a sua abundância, localização estratégica e envolvimento em processos fisiológicos e patológicos de doenças inflamatórias das vias aéreas, incluindo patologias respiratórias como doença pulmonar obstrutiva

crônica (DPOC), asma, câncer e fibrose cística (GRACE et al., 2013; NASSINI et al., 2012; WALLACE, 2017; XIA et al., 2018). Esses canais iônicos estão presentes em terminais nervosos vagais e possivelmente células auxiliares neuronais, podendo ser ativados por uma ampla variedade de estímulos e iniciar respostas reflexas como broncoespasmo e tosse em humanos e modelos animais (BELVISI; BIRRELL, 2017).

1.7.1 TRPV1

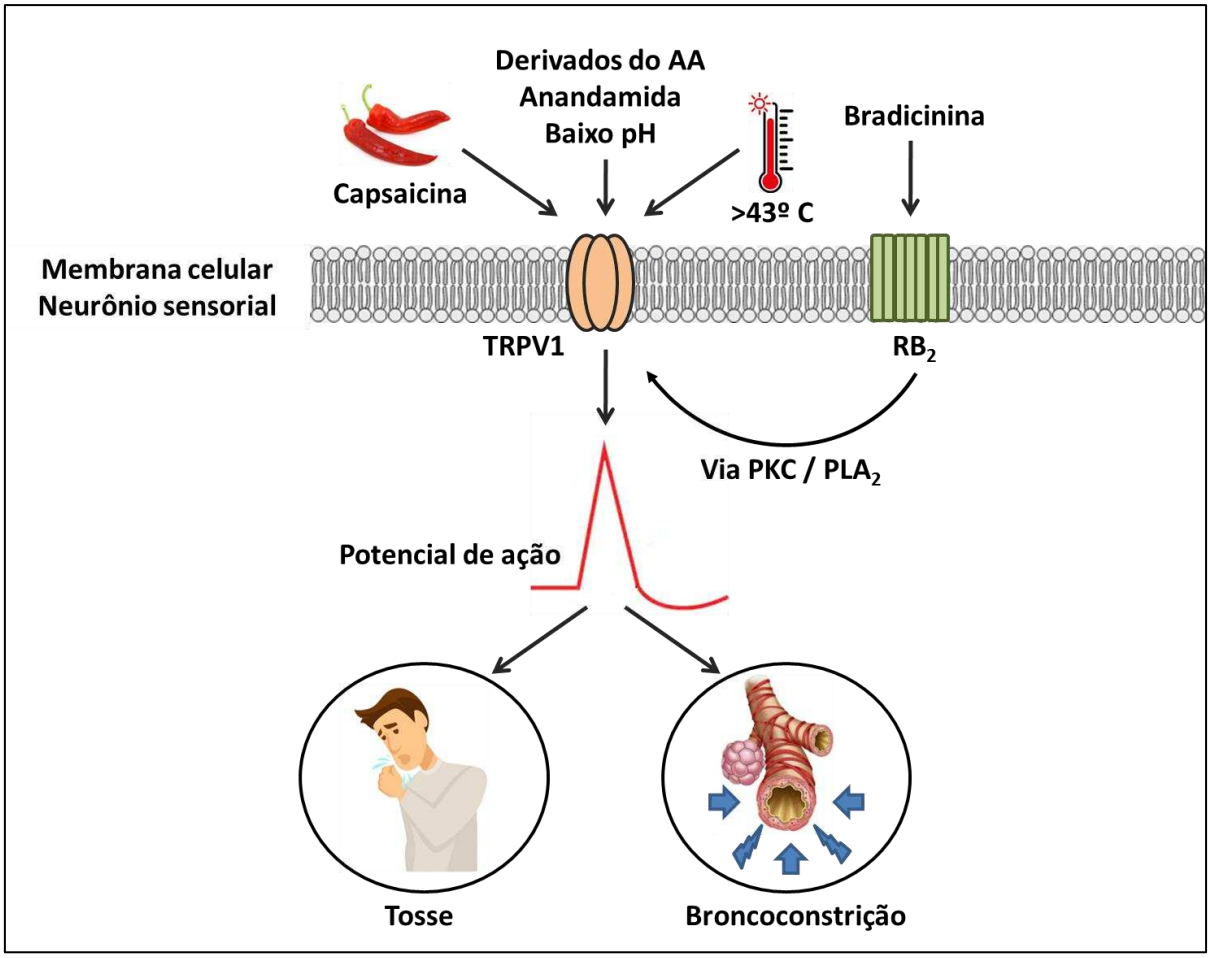
Os canais TRPV1 são os mais conhecidos dentre os TRPs. TRPV1 foi o primeiro membro da família de receptores vanilóides a ser caracterizado, clonado no ano de 1997 (CATERINA et al., 1997). São predominantemente expressos na membrana plasmática de fibras sensoriais aferentes do tipo C que inervam todo o trato respiratório, sendo que alguns desses neurônios co-expressam os canais TRPA1 ou TRPV4, e possuem habilidade de produzir neuropeptídeos sensoriais (DE LOGU et al., 2016; XIA et al., 2018; WALLACE, 2017; LEE; GU, 2009). Alternativamente, um pequeno número de fibras aferentes vagais do subtipo Aδ, que não expressam TRPV1 em condições normais, podem ter seu fenótipo alterado durante processos inflamatórios das vias respiratórias, fazendo com que a fibra expresse transientemente o canal (GEPPETTI; MATERAZZI; NICOLETTI, 2006; GRACE et al., 2013). Além disso, TRPV1 são também expressos na musculatura lisa pulmonar, no epitélio da traqueia, brônquios e laringe, células endoteliais vasculares e dendríticas do pulmão (ROGERIO; ANDRADE; CALIXTO, 2011; RADRESA et al., 2012).

TRPV1 forma predominantemente homotetrâmeros, embora heterotetrâmeros com canais TRPA1 e TRPV4 tenham sido reportados na literatura (NILIUS; SZALLASI, 2014). TRPV1 é conhecido por ser um canal polimodal, ativado por diversos estímulos, como por irritantes químicos, capsaicina e resiniferatoxina (CATERINA et al ., 1997; MEOTTI; DE ANDRADE; CALIXTO, 2014), baixo pH extracelular, anandamida, temperaturas quentes (> 42 ° C) e derivados do ácido araquidônico, AA) (GEPPETTI et al., 2010; GRACE et al., 2014).

Uma vez ativados, os canais proporcionam um aumento da concentração de cálcio intracelular e da excitabilidade das células, gerando potenciais de ação e promovendo a liberação de taquicininas e CGRP dos terminais nervosos. A

liberação axonal local dos neuropeptídeos age em células efetoras do trato respiratório induzindo broncoconstrição, extravasamento de plasma e quimiotaxia de células inflamatórias, respostas referidas como inflamação neurogênica (JIA; LEE, 2007; LEE; GU, 2009). Adicionalmente, reflexos centrais também são gerados como broncoconstrição e hipersecreção de muco, através de mecanismos colinérgicos (JIA; LEE, 2007; DU et al., 2019). Logo, o canal iônico TRPV1 possui um papel já estabelecido na tosse, e com frequência seus ligantes capsaicina e ácido cítrico são administrados como ferramenta para avaliação do reflexo da tosse em estudos clínicos e em animais (GRACE et al., 2011). As informações acima estão resumidas na FIGURA 3.

FIGURA 3 – RESUMO DA ATIVAÇÃO DO CANAL TRPV1 EM NEURÔNIOS SENSORIAIS E SUAS RESPOSTAS REFLEXAS NAS VIAS AÉREAS



Durante um cenário de doença respiratória, como asma e DPOC, vários mediadores inflamatórios produzidos pelo pulmão “doente” são liberados endogenamente como adenosina trifosfato (ATP), bradicinina, prostaglandinas, serotonina, fator de crescimento nervoso (NGF), quimiocinas, histamina ou proteases, que por meio da ativação de seus próprios receptores podem sensibilizar, diminuindo o limiar de ativação, e ativar indiretamente o TRPV1 (GRACE et al., 2014; BELVISI; BIRRELL, 2017; GOUIN et al., 2017). A ativação dos receptores B_2 por bradicinina, por exemplo, potencializou a atividade do TRPV1 em um sistema de expressão heterólogo (células HEK293) e em neurônios do gânglio da raiz dorsal (GRD) (CHUANG et al., 2001). Particularmente para esse mediador, o canal parece ser crucial para os seus efeitos farmacológicos decorrentes da excitação de fibras aferentes vagais das vias aéreas. Estudos em animais com deleção genética do receptor demonstraram que a ativação de fibras por bradicinina é diminuída na ausência de TRPV1, embora não seja totalmente extinguida (BESSAC; JORDT, 2008).

A sensibilização do canal é decorrente da ativação de proteínas cinases C e A (PKC e PKA) e proteína quinase dependente de Ca^{2+}/calmodulina (CaMKII). A atividade da PLC, que quando ativada diminui os níveis PIP2 e produz DAG, também está envolvida na sua sensibilização (CHUANG et al., 2001). Uma característica patofisiológica comum dessa sensibilização durante doenças inflamatórias das vias aéreas é a resposta sensorial exagerada conhecida como hipersensibilidade, caracterizada por irritação, dispneia, tosse e broncoconstrição a irritantes inalados (LEE; GU, 2009). De fato, estudos em cobaias e em pacientes afetados por asma, rinite, DPOC, tratados com IECAs, entre outras condições, demonstram uma maior sensibilidade a capsaicina, desencadeando respostas reflexas tussivas exageradas (GEPPETTI et al., 2010).

O envolvimento do TRPV1 nos efeitos adversos observados nas vias aéreas após o tratamento com IECA ainda não está totalmente elucidado. Recentemente demonstramos que o tratamento agudo com captopril induz extravasamento plasmático nas vias aéreas via sensibilização do TRPV1 por bradicinina (DE OLIVEIRA et al., 2016). Se TRPV1 está envolvido em outros efeitos adversos dos IECAs e se a continuidade do tratamento poderia alterar seu padrão de expressão nos pulmões ainda é uma questão a ser esclarecida. Estudos demonstraram que pacientes com doenças respiratórias podem apresentar maior expressão deste canal

e então, maior sensibilidade a estímulos como a capsaicina (XIA et al., 2018). Este efeito foi observado em um modelo de asma em ratos (BELVISI; BIRRELL, 2017), em um modelo animal submetido à inflamação alérgica (WATANABE et al., 2008), no epitélio das vias aéreas de pacientes asmáticos (MCGARVEY et al., 2014), e em pacientes com tosse crônica (GRONEBERG et al., 2004; MITCHELL et al., 2005; DU et al., 2019). Logo, a expressão deste canal pode ser regulada sob condições patológicas, servindo como explicação para a hipersensibilidade manifestada durante as condições inflamatórias (O'NEILL et al., 2012).

Nesse sentido, cada vez mais estudos sugerem o canal TRPV1 como alvo farmacológico atraente no tratamento de doenças respiratórias, focando principalmente na pesquisa de fármacos antitussígenos**.** Vários estudos pré-clínicos em diferentes modelos murinos demonstraram que o bloqueio ou a perda dos canais TRPV1 são eficazes no alívio da tosse, broncoconstrição, hiperresponsividade e inflamação das vias aéreas (BELVISI et al., 1992; LALLOO et al., 1995; TREVISANI et al., 2004; CHOI et al., 2018). Em cobaias, altas concentrações de capsazepina, um antagonista específico da ativação de fibras C induzidas por capsaicina, bloqueou a broncoconstrição e a tosse induzida por esse agonista (BELVISI et al., 1992; LALOO et al., 1995). Recentemente demonstramos que o tratamento intratraqueal com capsazepina foi capaz de diminuir o extravasamento plasmático induzido por captopril nas vias aéreas de ratos mecanicamente ventilados (DE OLIVEIRA et al., 2016). No entanto, capsazepina inibe não somente a ativação dos canais TRPV1, mas também possui ações não específicas, como a inibição de canais operados por voltagem (DOCHERTY; YEATS; PIPER, 1997), e então outros mecanismos adjacentes podem estar envolvidos.

Adicionalmente, observamos que o tratamento dos ratos no período neonatal com altas doses de capsaicina também foi capaz de inibir o extravasamento plasmático induzido por captopril nas vias aéreas (DE OLIVEIRA et al., 2016). De fato, já está bem estabelecido na literatura que a administração de uma ou duas doses de capsaicina, 50 mg/kg, nos primeiros dias de vida de ratos causa dessensibilização aguda, caracterizada por depleção de neuropeptídios e degeneração específica dos neurônios sensoriais periféricos que expressam TRPV1 (SCADDING, 1980; CZIKORA et al., 2013). Assim, o tratamento é capaz de desfuncionalizar seletivamente os neurônios sensoriais sensíveis a capsaicina, os levando a morte celular, possuindo um claro potencial terapêutico no manejo de

doenças em que essas vias neurais estão envolvidas (CZIKORA et al., 2013). Em humanos, a aplicação tópica repetida de capsaicina na mucosa nasal de pacientes com rinite não alérgica crônica, por exemplo, fez com que os pacientes apresentassem melhora dos sintomas, com função olfativa intacta e descontinuação do uso abusivo de vasocontritores nasais (GEPPETTI et al., 1988; LACROIX et al., 1991).

No entanto, com relação às doenças das vias aéreas de humanos, os estudos clínicos testando compostos antagonistas TRPV1 ainda são escassos (JIA; LEE, 2007). A maior preocupação quanto a administração de antagonistas desse canal é seu perfil de segurança, isso porque vários dos antagonistas avaliados causaram hipertermia, sugerindo que TRPV1 é tonicamente ativado nas vias termorregulatórias *in vivo* (GAVVA et al., 2008; LEHTO et al., 2008; NASRA; BELVISI, 2009). Assim, atualmente, as pesquisas buscam inibidores que não afetem a temperatura corporal (LEHTO et al., 2008). A expressão do canal TRPA1 é mais restrita, o que faz com que apresente um perfil de segurança interessante. Logo, é muito provável que a terapia ideal para os sintomas de doenças inflamatórias respiratórias, incluindo-se aqui os efeitos adversos induzidos por IECAs nas vias aéreas, envolva múltiplos TRPs (GRACE et al., 2011).

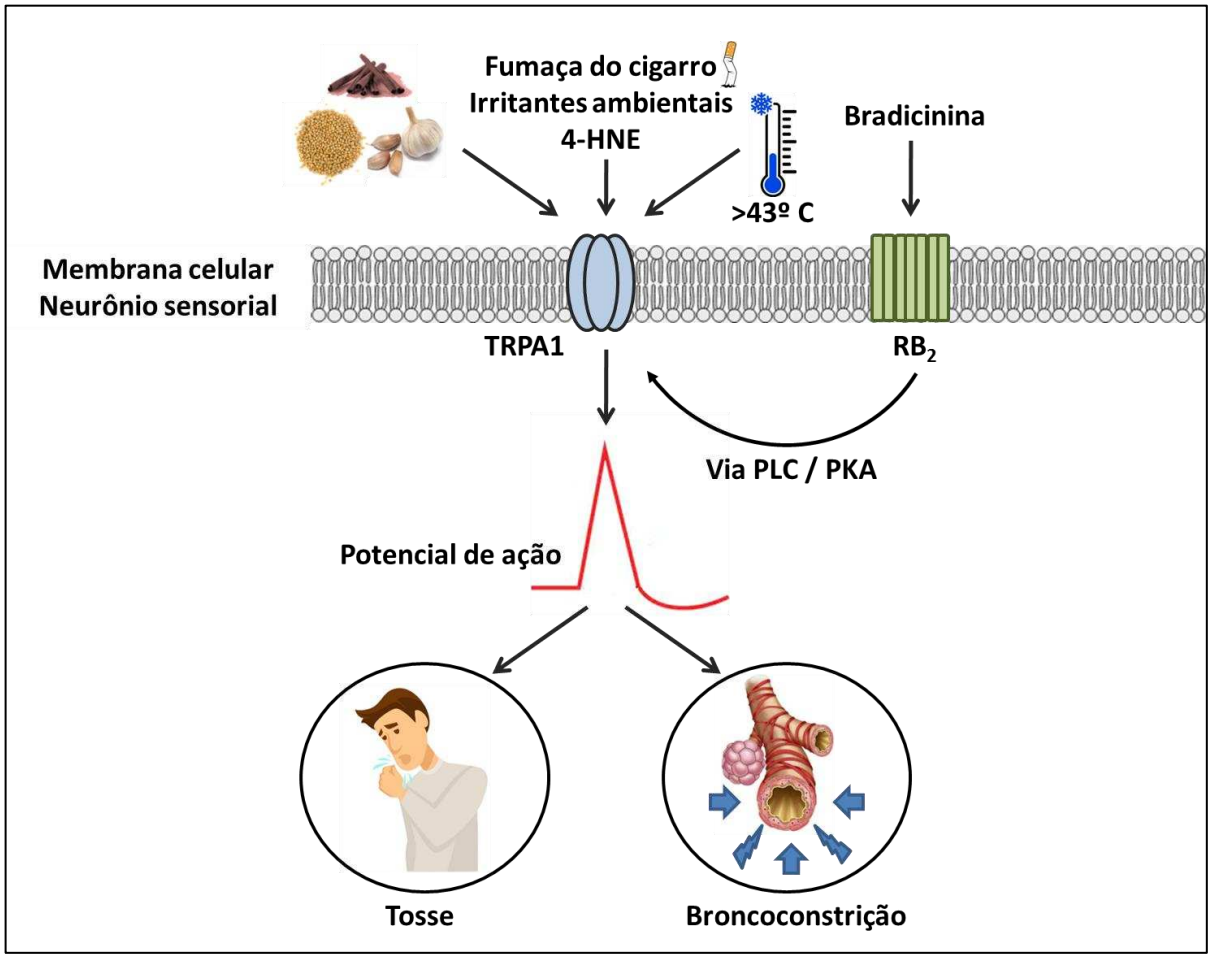
1.7.2 TRPA1

O canal TRPA1 é o único membro da família anquirina e foi primeiramente descoberto em uma cultura de fibroblastos do pulmão humano. O receptor é amplamente expresso em neurônios sensoriais vagais, onde está co-expresso com TRPV1 (PRETI; SZALLASI; PATACCHINI, 2012). TRPA1 parece ativar somente fibras C broncopulmonares vagais, desencadeando tosse, aumento da broncoconstrição e hiperreatividade brônquica em humanos e modelos animais (BONVINI; BELVISI, 2017).

O canal é ativado por uma série de irritantes exógenos. Nas vias aéreas de porquinhos da índia, isotiocianato de alila (presente na mostarda), cinamaldeído (da canela) e outros irritantes ambientais como acroleína e o crotonaldeído (presentes na fumaça do cigarro) foram capazes de induzir inflamação neurogênica e tosse (ANDRÈ et al., 2008; ANDRÈ et al., 2009). Há também evidências de que os canais TRPA1 são ativados por temperaturas frias abaixo de 17°C (STORY et al., 2003;

ZURBORG et al., 2007; CASPANI; HEPPENSTALL, 2009), embora esse achado seja questionado (ZHOU et al., 2011; GRACE et al., 2014), e calor nocivo (de modo dependente de uma tríade de canais TRPA1, TRPM3 e TRPV1) (VANDEWAUW et al., 2018). Espécies reativas de oxigênio (ROS) e de nitrogênio induzidas durante a lesão tecidual via peroxidação lipídica, como o 4-hidroxinonenal (4-HNE) também o ativam (GEPPETTI et al., 2010; XIA et al., 2018; BELVISI; BIRRELL, 2017) (FIGURA 4).

FIGURA 4 – RESUMO DA ATIVAÇÃO DO CANAL TRPA1 EM NEURÔNIOS SENSORIAIS E SUAS RESPOSTAS REFLEXAS NAS VIAS AÉREAS



De maneira semelhante ao canal TRPV1, a atividade do TRPA1 também é modulada por processos de dessensibilização e sensibilização (GRACE et al., 2013). A ativação repetida do receptor por estímulos químicos resulta na sua dessensibilização de modo Ca^{2+} dependente. Vários mediadores inflamatórios podem o sensibilizar, como fatores de crescimento, bradicinina, proteases via ativação de vários GPCRs ou receptores tirosina quinases (RTKs) através das vias adenosina 3',5'-monofosfato cíclico (AMPc) / PKA e PLC / PKC após a elevação de Ca^{2+} (BANDELL et al., 2004; GRACE et al., 2014; GOUIN et al., 2017). Uma vez ativado, os neurônios que expressam o canal liberam neuropeptídios pró-inflamatórios, iniciando a inflamação neurogênica (PRETI; SZALLASI; PATACCHINI, 2012).

Assim como o antagonismo do canal TRPV1, o bloqueio do TRPA1 em doenças inflamatórias das vias aéreas também tem despertado interesse. O antagonismo do canal TRPA1 inibiu a ativação de neurônios sensoriais vagais isolados de cobaia e a resposta tussiva induzida por cigarro, prostaglandina E2 (PGE_2) e bradicinina, e ao ser associado a um antagonista TRPV1, essas respostas foram abolidas (BENEMEI et al., 2015). O antagonista HC030031 bloqueou a inflamação aguda e a tosse induzida pela fumaça do cigarro e também a tosse induzida por agonistas TRPA1 (acroleina, cinamaldeido, isotiocianato de alila e crotonaldeido) em cobaias (ANDRÈ et al., 2009; CACERES et al., 2009). Em conjunto, os dados apresentados na literatura apoiam um papel dos antagonistas TRPA1 como medicamentos inovadores na intervenção farmacológica de doenças como asma e DPOC (MUKHOPADHYAY; KULKARNI; KHAIRATKAR-JOSHI, 2016). No entanto, estudos clínicos com antagonistas TRPA1 em pacientes humanos com doenças inflamatórias das vias aéreas ainda são escassos, assim como o seu papel no mecanismo pelo qual IECAs causam efeitos adversos nas vias aéreas.

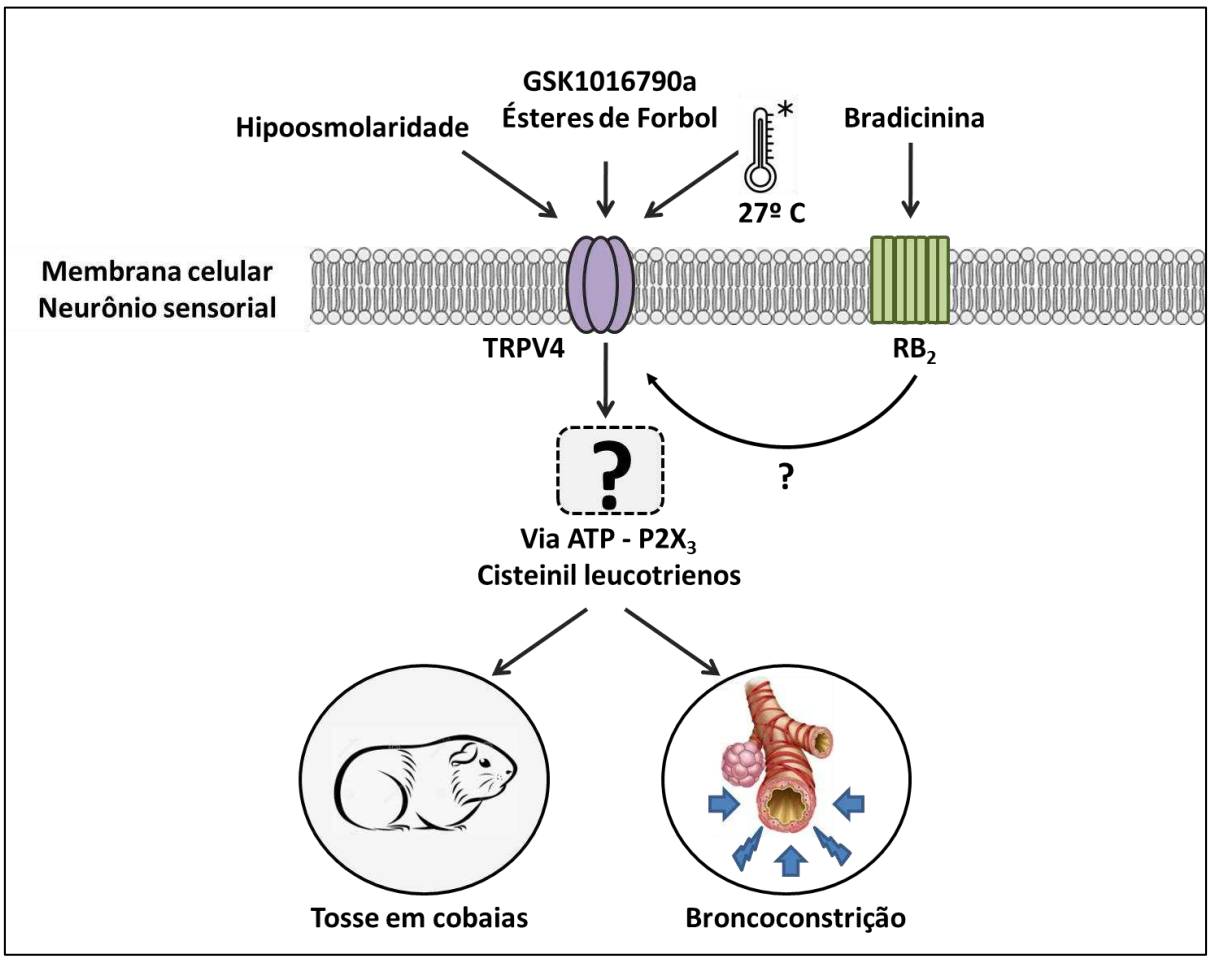
1.7.3 TRPV4

O TRPV4 é um canal catiônico não seletivo com alta permeabilidade ao Ca^{2+} e ao magnésio (Mg^{2+}) (GARCIA-ELIAS et al., 2014) e que, assim como os canais TRPV1 e TRPA1, participam de doenças inflamatórias do pulmão, como asma, DPOC e fibrose cística (BONVINI et al., 2016; SCHERAGA et al., 2017). Dentre os agonistas endógenos do canal estão temperatura entre 27–35°C (constitutivamente

ativo em temperaturas normais do corpo), ácidos epoxieicosatrienóicos, anandamida e estímulos hipotônicos (NILIUS; SZALLASI, 2014; GRACE et al., 2017). Produtos químicos também são potentes agonistas TRPV4 como ésteres de forbol e o agonista sintético GSK1016790A, ambos amplamente utilizados como ferramentas na investigação farmacológica e fisiológica dos canais, especialmente na regulação da permeabilidade vascular e integridade microvascular (WATANABE et al., 2002; WILLETTE et al., 2008; NILIUS; SZALLASI, 2014).

O canal TRPV4 está amplamente distribuído sendo detectado em células epiteliais dos brônquios humanos, células estruturais como musculatura lisa das vias aéreas, fibroblastos e vasos pulmonares, células inflamatórias como macrófagos e neurônios sensoriais (BELVISI; BIRRELL, 2017; XIA et al., 2018). Uma característica comum é a sua co-localização com TRPV1 em neurônios sensoriais que expressam neuropeptídios (BENEMEI et al., 2015). A estimulação do canal TRPV4 por GSK1016790A ou didecanoato de 4α-forbol (4α-PDD) induz disparos de neurônios sensoriais e tosse em cobaias, sendo, assim como os demais TRPs abordados anteriormente, alvos atrativos no tratamento da broncoconstrição e tosse (BENEMEI et al., 2015). No entanto, sua neurobiologia é distinta dos canais TRPV1 e TRPA1 e pouco se sabe sobre seu papel na ativação dos nervos sensoriais das vias aéreas (BONVINI et al., 2016). Seus agonistas e mecanismo de ativação/sensibilização estão resumidos na FIGURA 5.

FIGURA 5 – RESUMO DA ATIVAÇÃO DO CANAL TRPV4 EM NEURÔNIOS SENSORIAIS E SUAS RESPOSTAS REFLEXAS NAS VIAS AÉREAS



Os efeitos da ativação do canal TRPV4 a jusante de neurônios sensoriais nas vias aéreas ainda não foram totalmente elucidados (BONVINI et al., 2016).

Estudos demonstraram que a ativação do TRPV4 por seus agonistas promove um aumento do influxo de cálcio no gânglio nodoso de cobaias, acompanhado de despolarização de nervos vagais humanos, e ativação de fibras Aδ e não de fibras C, seguida de tosse em um modelo animal (BONVINI et al., 2016; BONVINI; BELVISI, 2017). Nas vias aéreas *in vivo*, através de técnicas de imagem de cálcio e eletrofisiologia, Bonvini e colaboradores (2016) identificaram uma via de sinalização TRPV4-ATP- receptores purinérgicos subtipo $P2X_3$ nesses nociceptores periféricos Aδ (BONVINI et al., 2016). Além disso, McAlexander e outros pesquisadores demonstraram que ativação do TRPV4 causa constrição de brônquios isolados das vias aéreas humanas, por um mecanismo dependente da produção de cisteinil leucotrienos (MCALEXANDER et al., 2014). Logo, o antagonismo do canal também seria um alvo farmacológico atrativo, especialmente

por envolver uma população diferente de fibras aferentes das vias aéreas e possuir mecanismos para a sua ativação diferentes dos canais TRPV1 e TRPA1 (BONVINI et al., 2015).

2 PLANO DA TESE

2.1 JUSTIFICATIVA

O uso de IECAs continua a aumentar em todo o mundo, e então, maior é a probabilidade dos pacientes desenvolverem os efeitos adversos desta classe sobre as vias respiratórias. Uma vez que a terapia farmacológica disponível para o tratamento desses efeitos é escassa e seu mecanismo patofisiológico não está totalmente elucidado é imperativo investigar novos mecanismos de modulação destes efeitos e apontar novos alvos terapêuticos para auxiliar a tomada de decisões. Evidências demonstraram que os canais iônicos TRPV1, TRPA1 e TRPV4 são alvos farmacológicos potencialmente importantes para o tratamento de sintomas de algumas das principais doenças respiratórias devido a sua abundância, localização estratégica e função nas vias aéreas. Nesse sentido, investigamos o papel desses canais nos efeitos adversos induzidos por captopril, um IECA, nas vias aéreas de ratos, visando o fornecimento de informações para nortear estratégias terapêuticas efetivas no futuro.

2.2 OBJETIVO GERAL

Investigar se canais iônicos TRPs estão envolvidos nas alterações das vias aéreas de ratos induzidas por captopril.

2.3 OBJETIVOS ESPECÍFICOS

- Avaliar farmacologicamente o papel dos canais TRPV1, TRPA1 E TRPV4, na hiperresponsividade brônquica e extravasamento plasmático induzido pelo tratamento com captopril, usando agonistas e antagonistas desses canais;

- Avaliar se a dessensibilização de neurônios sensoriais das vias aéreas, onde os canais TRPV1, TRPA1 E TRPV4 estão co-expressos, amenizam as respostas inflamatórias induzidas pelo IECA;

- Identificar se a bradicinina atua na via de sinalização pela qual captopril sensibiliza esses canais;

- Identificar possíveis diferenças entre a via de administração (intravenosa e oral) e o tempo de tratamento (agudo e subcrônico) na broncoconstrição e inflamação pulmonar induzida por captopril;

- Avaliar possíveis alterações histológicas e fenotípicas nos pulmões de ratos submetidos a diferentes regimes de tratamento com captopril;

Todos os protocolos experimentais e a análise estatística empregada nesta tese estão descritos de maneira detalhada nos artigos científicos que a compõe. No entanto, resumidamente, os objetivos foram alcançados empregando:

- Um modelo animal *in vivo* (ratos Wistar machos) para avaliação da resistência das vias aéreas e do extravasamento plasmático, de modo a permitir a investigação direta do envolvimento dos TRPV1, TRPA1 e TRPV4 através de ferramentas farmacológicas;

- A metodologia de contagem total de leucócitos no lavado broncoalveolar;

- A técnica de histologia para investigar alterações inflamatórias nos pulmões;

- A técnica de imunohistoquímica para investigar alterações nos padrões fenotípicos nos pulmões;

3 ARTIGOS CIENTÍFICOS

3.1 MANUSCRITO ORIGINAL 1

Estruturado e enviado para publicação na revista *Pulmonary Pharmacology and Therapeutics*

THE ROLE OF TRPA1 AND TRPV4 CHANNELS IN BRONCHOCONSTRICTION AND PLASMA EXTRAVASATION IN AIRWAYS OF RATS TREATED WITH CAPTOPRIL

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ABSTRACT

Angiotensin-converting enzyme inhibitors (ACEis) may cause adverse airway events, such as cough and angioedema, due to a reduction in bradykinin breakdown and consequent activation of bradykinin type 2 receptor (B_2 receptor). However, it is also documented that bradykinin can sensitize transient receptor potential ankyrin 1 (TRPA1) and vanilloid 4 (TRPV4), which are implicated in several inflammatory airway diseases. Based on these considerations, the aim of this study was to understand the role of TRPA1 and TRPV4 channels in the bronchoconstrictive response and plasma extravasation in the trachea of rats pretreated with captopril. Using methods to detect alterations in airway resistance and plasma extravasation, we found that intravenous (i.v.) administration of bradykinin (0.03–0.3 μmol/kg), allyl isothiocyanate (100–1000 μmol/kg) or GSK1016790A (0.01–0.1 μmol/kg), but not des-arg9-bradykinin (DABK; 100–300 μmol/kg), induced bronchoconstriction in anaesthetized rats. In doses that did not cause significant bronchoconstriction, bradykinin (0.03 μmol/kg) or allyl isothiocyanate (100 μmol/kg), but not GSK1016790A (0.01 μmol/kg) or DABK (300 μmol/kg) induced an increased bronchoconstrictive response in rats pretreated with captopril (2.5 mg/kg, i.v.). On the other hand, in rats pretreated with captopril (5 mg/kg, i.v.), an increased bronchoconstrictive response to GSK1016790A (0.01 μmol/kg) was observed. The bronchoconstrictive response induced by bradykinin in captopril-pretreated rats was inhibited by intratracheal treatment (i.t.) with HC030031 (300 μg/50 μl; 36 ± 9%) or HC067047 (300 μg/50 μl; 35.1 ± 16%), for TRPA1 and TRPV4 antagonists, respectively. However, the co-administration of both antagonists did not increase this inhibition. The bronchoconstriction induced by allyl isothiocyanate in captopril-pretreated rats (2.5 mg/kg) was inhibited (58.3 ± 8%) by the B_2 receptor antagonist

HOE140 (10 nmol/50 µl, i.t.). Similarly, the bronchoconstriction induced by GSK1016790A in captopril-pretreated rats (5 mg/kg) was also inhibited (84.2 ± 4%) by the B_2 receptor antagonist HOE140 (10 nmol/50 µl, i.t.). Furthermore, the plasma extravasation induced by captopril on the trachea of rats was inhibited by pretreatment with HC030031 (47.2 ± 8%) or HC067047 (38.9 ± 8%). Collectively, these findings support the hypothesis that TRPA1 and TRPV4, via a B_2 receptor activation-dependent pathway, are involved in the plasma extravasation and bronchoconstriction induced by captopril, making them possible pharmacological targets to prevent or remediate ACEi-induced adverse respiratory reactions.

Keywords: TRPA1; TRPV4; captopril; bradykinin

Abbreviations: ACEis: angiotensin-converting enzyme inhibitors; ACE: angiotensin-converting enzyme; ANOVA: analysis of variance; ATP: adenosine triphosphate; B1 receptor: bradykinin receptor type 1; B_2 receptor: bradykinin receptor type 2; DABK: des-arg9-bradykinin; DMSO: dimethyl sulfoxide; Fig.: figure; i.p.: intraperitoneal administration; i.t.: intratracheal administration; i.v.: intravenous administration; NaCl: Sodium chloride; P2X3: P2X purinoceptor 3; s.e.m.: standard error of the mean; TRPA1: transient potential receptor ankyrin 1; TRPV1: transient potential receptor vanilloid 1; TRPV4: transient potential receptor vanilloid 4.

1. Introduction

Although studies underline that angiotensin-converting enzyme inhibitors (ACEis) cause adverse airway events due to a reduction in bradykinin breakdown and consequent activation of bradykinin type 2 receptor (B2 receptor) [1], recently, de Oliveira and collaborators suggested that transient receptor potential vanilloid 1 (TRPV1) could also be involved in these actions [2]. In general, TRPV1, TRP ankyrin 1 (TRPA1) and TRP vanilloid 4 (TRPV4) channels acts as molecular integrators of multiple types of noxious stimuli and are co-expressed in primary sensory afferents of airways, playing a role in physiological and pathological processes [3–6]. However, it has not yet been investigated whether TRPA1 and TRPV4 are also involved in the adverse effects in airways induced by ACEis. Similar to TRPV1, TRPA1 and TRPV4 can be sensitized by bradykinin in in vitro and in vivo experiments [7–9]. In fact, activation of the B2 receptor by bradykinin mediates the release of key intracellular messengers to sensitize TRPA1 and TRPV4 [10–12].

TRPA1 is an ion channel known to be activated by constituents of air pollution, pungent ingredients such as isothiocyanates, oxidation, noxious cold and acute noxious heat sensation [13–15]. In the airways, several compounds have been demonstrated to stimulate TRPA1 and induce neurogenic inflammation, such as N-acetyl-p-benzo-quinoneimine, a metabolite of acetaminophen, cigarette smoke and environmental pollutants such as crotonaldehyde and acrolein [16, 17]. Besides bradykinin, TRPA1 activity can be modulated by other endogenous activators including reactive oxygen and nitrogen species induced during tissue damage via lipid peroxidation, such as 4-hydroxy-2-nonenal, 5,6-epoxyeicosatrienoic acid, 15-deoxy-Δ12,14-prostaglandin J2 and nitrooleic acid [6, 13, 18, 19]. In addition, intracellular calcium and the activation of protease-activated receptor 2 also modulate the channel [18].

While TRPA1 activation releases pro-inflammatory sensory neuropeptides of vagal bronchopulmonary C-fibres, leading to cough, bronchoconstriction and bronchus hyperreactivity in humans and animal models [7, 14, 20], TRPV4 also promotes similar responses but by a different mechanism [21, 22]. The TRPV4 channel is activated by osmotic changes (hypotonicity), moderate temperatures (>24 °C), mechanical perturbations, pH, arachidonic acid metabolites, moderate heat, phorbol ester (4α-PDD) and by GSK1016790A, a selective and potent agonist widely used as a pharmacological tool [21, 23–25]. Studies have demonstrated that TRPV4 activation leads to firing of guinea pig A□-fibres but not C-fibres [7]. This distinct neurobiology seems to involve the TRPV4–ATP–P2X3 signalling pathway [7]. McAlexander and collaborators also showed that TRPV4 activation contracts the human bronchus by a mechanism depending on the production of cysteinyl leukotrienes [22]. For this reason, the TRPV4 blockade has also been considered a differentiated target in the treatment of lung diseases [21]. Based on these considerations, the aim of this study was to understand the role of TRPA1 and TRPV4 in the bronchoconstrictive response and plasma extravasation in the trachea of rats pretreated with captopril.

2. Material and methods

2.1 Animals

Male adult Wistar rats weighing 250 - 300g were used in our experiments. A maximum of four rats were group-housed. Animals were maintained in a room with a controlled temperature (21 ± 2 ºC) under a 12 h light/dark cycle (lights on at 6 a.m.). Food and water were provided *ad libitum*. Rats were randomly assigned before

treatment and the number of animals used in the study was the minimum necessary to obtain consistent data. This study is in compliance with the ARRIVE guidelines, as previously reported by Kilkenny and collaborators [26], and all experimental procedures were approved by the Institutional Committee for Animal Care and Use of Federal University of Paraná (Protocols number 800/2016 and 1160/2018). A common procedure in all protocols was intraperitoneal anaesthesia with ketamine (50 mg/kg) and xylazine (10 mg/kg). After induction of anaesthesia, a surgical procedure was performed to insert a cannula into the cervical portion of the trachea of the rats. The cannula was then fixed in place with suture wire and connected to artificial ventilation using room air (Ugo Basile Rodent Ventilator model 7025; 50 strokes/min; 10 ml/kg of room air; Ugo Basile, Comerio, Varese, Italy) [2, 27].

2.2 Evaluation of bronchoconstrictive response in captopril-pretreated animals

The bronchoconstrictive response was evaluated using methods to measure airway resistance. A bronchospasm transducer (Ugo Basile Bronchospasm Transducer 17020, Ugo Basile, Italy) was used, following the Konzett and Rossler air overflow technique [28] and Amdur and Mead method [29] in anaesthetized rats. The transducer was connected to a data acquisition system (DataCapsule-Evo Digital Recorder 17308, Ugo Basile, Italy) with LabScribe3TM recording and analysis software onboard. The anaesthetized animals underwent a stabilization period of at least 10 min before the treatments and the evaluation. The results were expressed as the increase in airway resistance (volume-displacement of H2O/ml) above the baseline value that was measured prior to drug administration, a measure of bronchoconstriction [30].

Initially, we performed dose–response curves for the B_1 receptor, B_2 receptor, TRPA1 or TRPV4 agonists. Specifically, intravenous (i.v.) doses of des-arg9-bradykinin

(DABK; 100–300 μmol/kg; a B1 receptor agonist) [31], bradykinin (0.03–0.3 μmol/kg; a B_2 receptor agonist) [32–34], allyl isothiocyanate (100–1000 μmol/kg; a TRPA1 agonist) [16, 20, 35] and GSK1016790A (0.01–0.1 μmol/kg; a TRPV4 agonist) [36, 37] were injected into animals not treated with captopril. In order to evaluate whether doses of the agonists incapable of promoting a bronchoconstrictive response, per se, could promote bronchoconstriction in the captopril-pretreated rats, DABK (300 μmol/kg), bradykinin (0.03 μmol/kg), allyl isothiocyanate (100 μmol/kg) or GSK1016790A (0.01 μmol/kg) was injected (i.v.) 10 min after captopril administration (2.5 mg/kg, i.v.) [2, 38]. The choice of the dose of captopril was based on previous data described in the literature in the plasma extravasation model [2, 38]. Next, using the same experimental protocol, captopril (5 mg/kg, i.v.) was administered; 10 min after that the animals received a GSK1016790A injection (0.01 μmol/kg, i.v.) and the bronchoconstrictive response was evaluated. In another set of experiments, the TRPA1 antagonist HC030031 (300 μg/50 μl) and TRPV4 antagonist HC067047 (300 μg/50 μl) [16, 39], separately or in association, the B_2 receptor antagonist HOE140 (10 nmol/50 μl) [2] or their respective vehicles were administered intratracheally (i.t.) 15 min prior to captopril (2.5 or 5 mg/kg). Ten minutes after the administration of captopril, bradykinin (0.03 μmol/kg; i.v.), allyl isothiocyanate (100 μmol/kg; i.v.) or GSK1016790A (0.01 μmol/kg; i.v.) was administered and the bronchoconstrictive response was evaluated.

2.3 Plasma protein extravasation

In order to investigate the roles of TRPA1 and TRPV4 channels in captopril-induced plasma extravasation in the airways of rats, the animals were first pretreated with the selective TRPA1 antagonist HC030031 (300 μg/50 μl), the TRPV4 antagonist HC067067 (300 μg/50 μl) or vehicle (0.9% NaCl composed of 7.5% DMSO and 7.5%

Tween 80) via the i.t. route. Evans Blue dye (30 mg/kg, i.v.) and captopril (2.5 mg/kg, i.v.) were administered 15 min after the antagonist pretreatment [2]. Ten minutes after administration of captopril, transcardiac perfusion with 0.9% NaCl was performed by inserting a cannula into the left ventricle directed towards the aorta in rats previously anaesthetized. The tracheae of the animals were removed, cleaned of connective tissues, washed and weighed and then incubated in 1 ml of formamide for dye extraction. The samples were kept for approximately 24 h at room temperature in the dark. The amount of dye extracted was measured by a spectrophotometer (620 nm) and interpolated on a standard dilution curve, expressing the data as micrograms of dye per gram of tissue (μg/g) [2, 40].

2.4 Drugs and reagents

The following drugs were used: allyl isothiocyanate, bradykinin, captopril, DABK, Evans blue dye, GSK1016790A, HC030031, HC067047 and HOE140, all of which were purchased from Sigma Chemical Co., St. Louis, USA. Evans blue dye, bradykinin, captopril and HOE140 were prepared in 0.9% NaCl. The allyl isothiocyanate and GSK1016790A solutions were made using 0.9% NaCl containing 0.5%-1% of dimethyl sulfoxide (DMSO). HC030031 and HC067047 solutions were made using 0.9% NaCl composed of 7.5% DMSO and 7.5% Tween 80. The solutions were diluted on the day of the experiment just prior to use.

2.5 Statistical analysis

Results are reported as the mean ± standard error of mean (s.e.m.). The number of animals for each experimental group (*n*) is described in detail in the figure legends and the necessary sample size was previously calculated using GPower 3.1. The

normality assumption was tested by Shapiro-Wilk Normality test. Statistical significance between the groups was assessed by means of one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls test or by unpaired Student's t test, using GraphPad Prism software, versions 5.01 and 6.01. For all comparisons, values of $P \leq 0.05$ were considered statistically significant.

3. Results

3.1 Characterization of the bronchoconstrictive responses induced by B_1 receptor, B_2 receptor, TRPA1 and TRPV4 agonists

As shown in Fig. 1, the administration of bradykinin (0.03–0.3 μmol/kg; Fig. 1A), allyl isothiocyanate (100–1000 μmol/kg; Fig. 1B) or GSK1016790A (0.01–0.1 μmol/kg; Fig. 1C) induced a bronchoconstrictive response in a dose-dependent manner as compared with the vehicle-treated control group.

In contrast, the B_1 receptor agonist DABK did not cause significant bronchoconstriction at the doses tested (100–300 μmol/kg; i.v.; data not shown). The dose of each agonist that did not promote bronchoconstriction, *per se*, was selected for the next group of experiments.

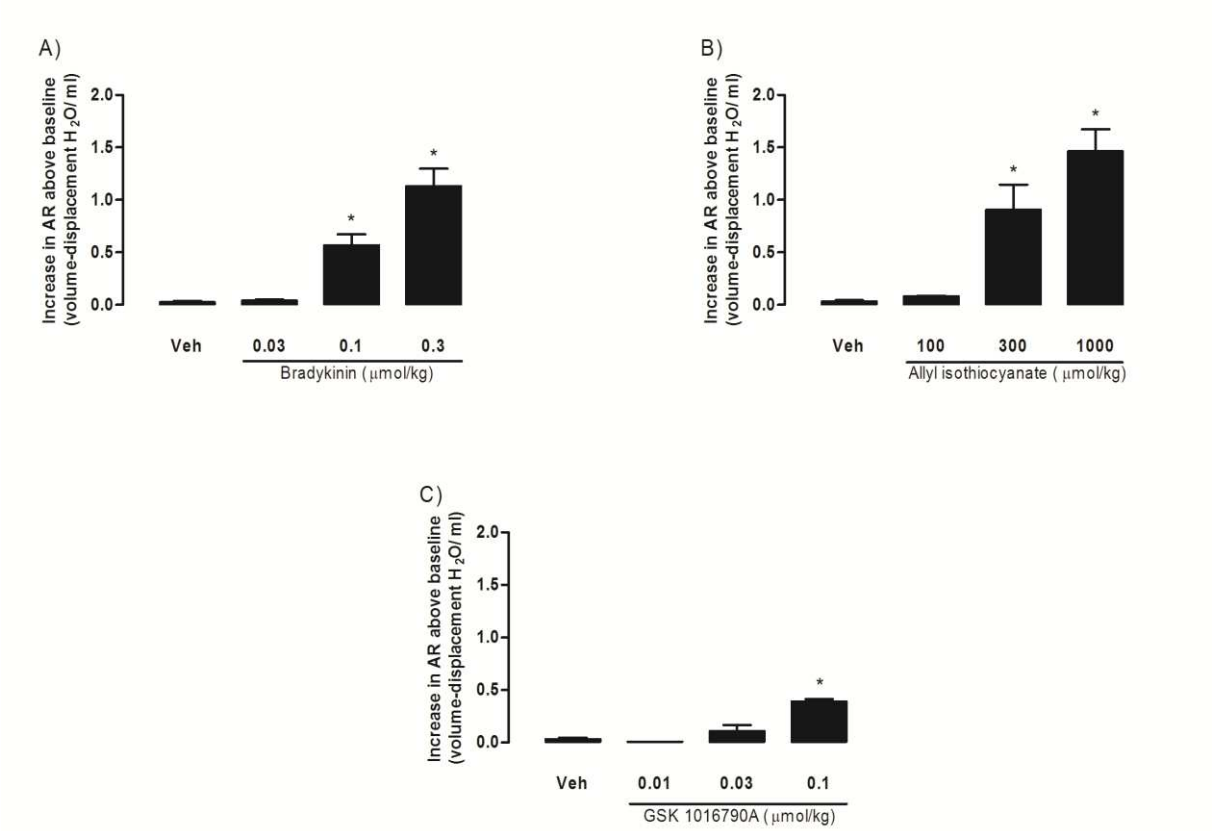

Fig. 1: Effects of increasing doses of intravenous bradykinin (A; 0.03 – 0.3 μmol/kg; B_2 receptor agonist), allyl isothiocyanate (B; 100 – 1000 μmol/kg; TRPA1 agonist) or GSK1016790A (C; 0.01 – 0.1 μmol/kg; TRPV4 agonist) on rat airways. Airway resistance (AR) is expressed as the increase in AR above the baseline value measured prior to drug administration. *denotes a significant increase in AR and significant difference from the vehicle-treated group (Veh), corresponding to $P \leq 0.05$ (one-way ANOVA followed by Student-Newman-Keuls test). Veh (vehicle): $n = 7$; Bradykinin 0.03, 0.01 and 0.3 μmol/kg: $n = 8$ for each group; Allyl isothiocyanate 100, 300 and 1000 μmol/kg: $n = 7$ for each group; GSK1016790A 0.01, 0.03 and 0.1 μmol/kg: $n = 8$ for each group.

3.2 Characterization of the bronchoconstrictive responses induced by B_1 receptor, B_2 receptor, TRPA1 and TRPV4 agonists in captopril-pretreated rats

In a different set of experiments, in doses that did not cause significant bronchoconstriction, bradykinin (0.03 μmol/kg, i.v., Fig. 2A) or allyl isothiocyanate (100 μmol/kg, i.v., Fig. 2B), but not GSK1016790A (0.01 μmol/kg, i.v., Fig. 2C) or DABK (300 μmol/kg, i.v., data not shown) induced an increased bronchoconstrictive

response in rats pretreated with captopril (2.5 mg/kg, i.v.). On the other hand, the pretreatment with captopril (5 mg/kg, i.v.) was able to increase the bronchoconstriction induced by GSK1016790A (0.01 µmol/kg, i.v.) (Fig. 2D).

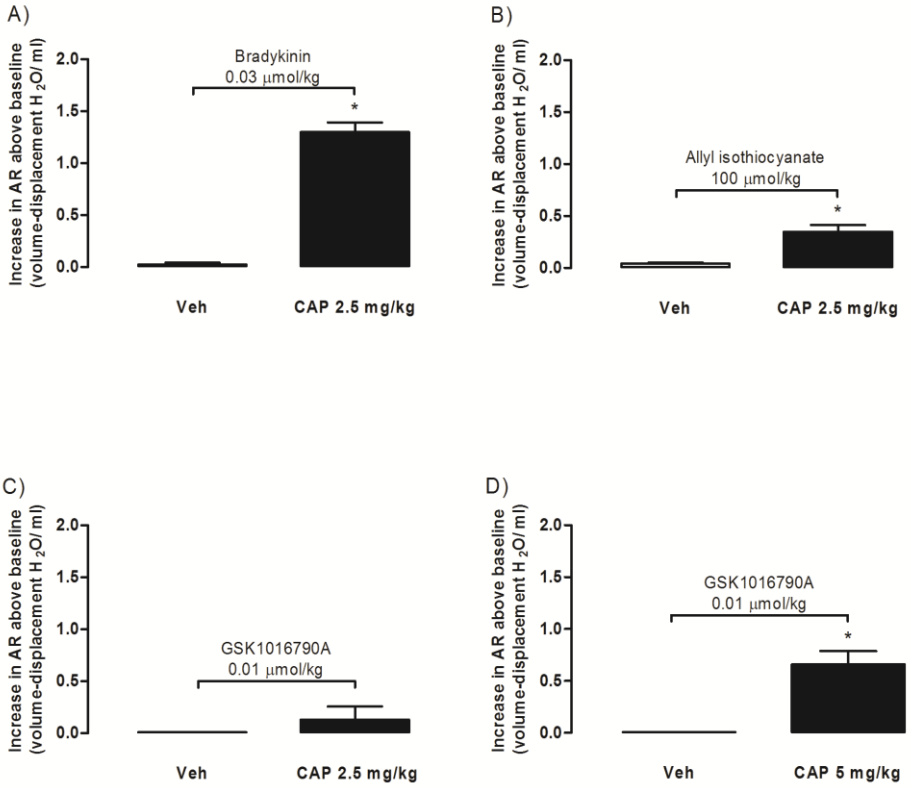

Fig. 2: Effects of acute treatment of captopril on rat airway resistance. Bradykinin (0.03 µmol/kg, A), allyl isothiocyanate (100 µmol/kg, B) or GSK1016790A (0.01 µmol/kg, C and D) were injected 10 min after intravenous pretreatment with 2.5mg/kg or 5 mg/kg captopril (CAP, D). Airway resistance (AR) is expressed as the increase in AR above the baseline value measured in the groups treated with the agonist plus saline (captopril vehicle). * denotes a significant difference ($P \leq 0.05$) from the vehicle-treated group (Veh) (unpaired Student's *t* test). Veh (vehicle): $n = 7$; Bradykinin + CAP 2.5 mg/kg: $n = 7$; Allyl isothiocyanate + CAP 2.5 mg/kg: $n = 8$; GSK1016790A + CAP 2.5 mg/kg: $n = 7$; GSK1016790A + CAP 5 mg/kg: $n = 7$.

3.3 Effects of B_2 receptor, TRPA1 and TRPV4 antagonists on the bronchoconstrictive responses induced by bradykinin, allyl isothiocyanate or GSK1016790A in captopril-pretreated rats

The bronchoconstriction induced by bradykinin (0.03 μmol/kg, i.v.) in captopril-pretreated rats (2.5 mg/kg, i.v.) was significantly inhibited by i.t. administration of HC030031 (300 μg/50 μl; Fig. 3A) or HC067047 (300 μg/50 μl; Fig. 3B). The combined administration of HC030031 plus HC067047 did not potentiate this effect (Fig. 3C). In addition, HOE140 (10 nmol/50 μl, i.t.) markedly prevented the bronchoconstriction induced by allyl isothiocyanate (100 μmol/kg, i.v., Fig. 4A) or GSK1016790A (0.01 μmol/kg, i.v., Fig. 4B) in animals pretreated with captopril (2.5 or 5 mg/kg, i.v., respectively).

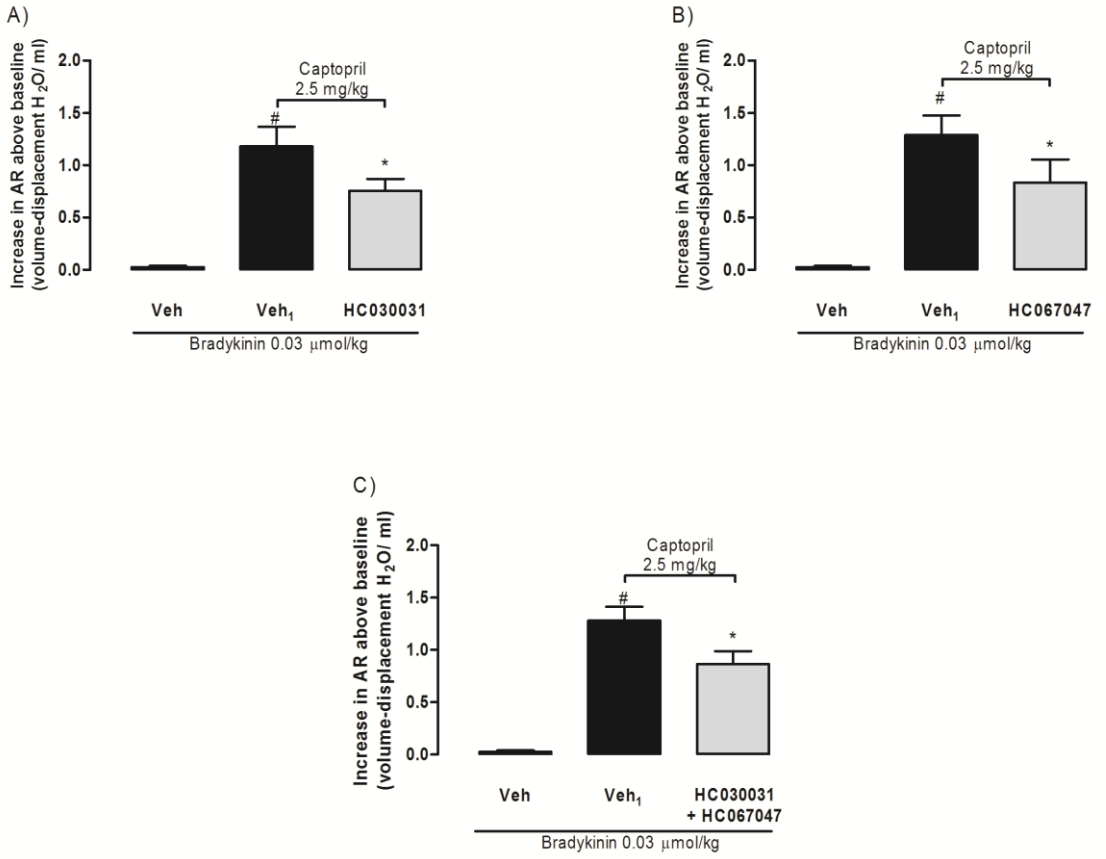

Fig. 3: Effects of TRPA1 or TRPV4 antagonism on the increased airway resistance (AR) induced by bradykinin (0.03 µmol/kg) after the acute pre-administration of captopril (2.5 mg/kg). The TRPA1 antagonist HC030031 (300 µg/50 µl, A) and TRPV4 antagonist HC067047 (300 µg/50 µl, B), separately and combined (C), were given intratracheally 15 min before captopril intravenous pre-treatment. Bradykinin injection was performed 10 min after. * denotes a significant difference ($P \leq 0.05$) from the vehicle-pretreated group 15 min before captopril and bradykinin injection (Veh_1). # denotes a significant difference from the vehicle-pretreated group 15 min before pre-injection of captopril vehicle and bradykinin (Veh) (one-way ANOVA followed by Student-Newman-Keuls test). Veh (vehicle): $n = 7$; Veh_1: $n = 7$; HC030031 group: $n = 7$; HC067047 group: $n = 7$; HC030031 + HC067047 group: $n = 7$.

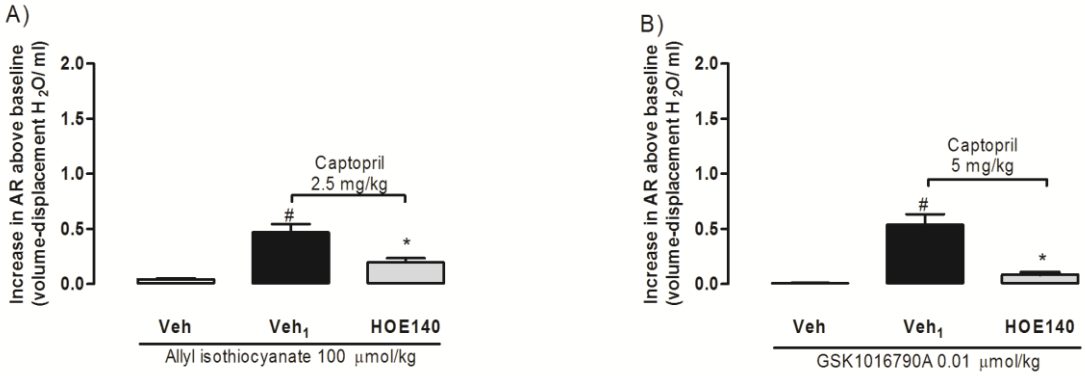

Fig. 4: Effects of B_2 receptor antagonism on the increased airway resistance (AR) induced by allyl isothiocyanate (100 µmol/kg) or GSK1016790A (0.01 µmol/kg) after acute pretreatment of captopril (2.5mg/kg or 5mg/kg, respectively). HOE140 (10 nmol/50 µl) was given intratracheally 15 min before captopril intravenous pretreatment. Ten minutes after the ACE inhibitor treatment, an injection of allyl isothiocianate or GSK1016790A were performed. * denotes a significant difference ($P \leq 0.05$) from the vehicle-pretreated group 15 min before injection of captopril (Veh_1). # denotes a significant difference from the vehicle-pretreated group 15 min before injection of captopril vehicle (Veh) (one-way ANOVA followed by Student-Newman-Keuls test). Veh: $n = 7$; Veh_1: $n = 7$; HOE140 $n = 7$.

3.4 Effects of TRPA1 and TRPV4 antagonists on plasma extravasation in the tracheae of captopril-pretreated rats

The administration of captopril (2.5 mg/kg, i.v.) induced significant increases in plasma extravasation in the trachea (118.3 ± 16.7 µg/g) of rats when compared with the vehicle-treated group. Pretreatment with HC030031 (300 µg/50 µl, i.t.) 15 min prior to captopril treatment markedly inhibited this plasma extravasation (62.4 ± 10 µg/g; Fig. 5A) when compared to vehicle-pretreated rats. Similarly, captopril-induced plasma extravasation in the trachea was also effectively attenuated by i.t. pretreatment with HC067047 (300 µg/50 µl) 15 min prior to captopril administration, when compared to vehicle-pretreated rats (72.2 ± 10.4 µg/g; Fig. 5B).

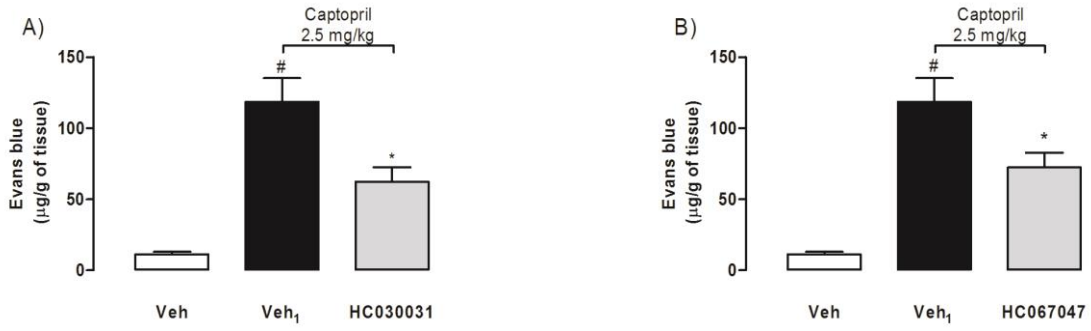

Fig. 5: Effects of TRPA1 and TRPV4 antagonism on the airway plasma extravasation induced by captopril. HC030031 (300 µg/50 µl, A) or HC067047 (300 µg/50 µl, B) was given intratracheally 15 min before intravenous pretreatment of Evans blue dye and captopril (2.5mg/kg; administered immediately after Evans blue). The trachea was excised 10 min later. * denotes a significant difference ($P \leq 0.05$) from the vehicle-pretreated group 15 min before captopril (Veh1). # denotes a significant difference from the vehicle-pretreated group 15 min before captopril vehicle (Veh) (one-way ANOVA followed by Student-Newman-Keuls test). Veh: n = 10; Veh1: n = 10; HC030031: n = 10; HC067047: n = 10.

4. Discussion

In the present study, we have demonstrated that bradykinin induces bronchoconstriction in rat airways. These data are consistent with previous studies reported by Greenberg et al. and Lau et al. that showed the same responses in guinea pig airways [32, 41]. On the other hand, our data also demonstrate that administration of the selective B_1 receptor agonist DABK does not elicit bronchoconstriction (data not shown). It is well known that B_1 receptor, unlike B_2 receptor, is normally absent under physiological conditions, a fact that could explain the lack of responsiveness to DABK [42, 43]. However, these results further reinforce the notion that bradykinin plays a crucial role in airway function.

Our results also showed the importance of TRPA1 and TRPV4 in airway function. Previous data have demonstrated the role of these receptors in the respiratory tract [19] and, here, we demonstrated in an animal model that these channels are also involved in the bronchoconstrictive response. We found that allyl isothiocyanate and GSK1016790A, TRPA1 and TRPV4 channel agonists, respectively, promoted bronchoconstriction in rats in a dose-dependent manner, thus confirming that both receptors are able to modulate airway alterations.

In addition, we also investigated when these channels could be involved in the adverse respiratory reactions induced by ACEis therapy. First, we demonstrated that the captopril does not promote bronchoconstriction in anaesthetized ventilated rats (data not shown) but does appear to mediate alterations on the respiratory tract via modulation of B_2 receptor. In fact, here we observed that captopril pretreatment increased the bronchoconstrictive response to bradykinin, an effect that was inhibited by the B_2 receptor antagonist.

Interestingly, captopril also increased the bronchoconstrictive response induced by a low dose of allyl isothiocyanate, a TRPA1 agonist. Considering that the TRPA1 channel is co-expressed with B_2 receptor in peptidergic primary afferent fibres [44], it is possible that a reduction in bradykinin breakdown, promoted by ACE inhibition, could modulate the TRPA1 in the airways. In fact, TRPA1 may be sensitized by bradykinin through activation of B_2 receptor [8, 45, 46]. Therefore, in this study, bradykinin could have phosphorylated the TRPA1 channel, reducing its activation threshold and thus resulting in bronchoconstriction.

Similarly, studies have shown that TRPV4 activation may be potentiated by bradykinin in HEK 293 cells [11] and that bradykinin can also sensitize TRPV4 to

induce mechanical hyperalgesia in mice [9]. In this study, we observed that treatment with 5 mg/kg captopril, but not 2.5 mg/kg, increased the bronchoconstriction induced by GSK1016790A stimulus. This result supports the hypothesis that the captopril effect on enhancing bronchoconstriction occurs in a dose-dependent manner and that bradykinin plasma levels after treatment with 2.5 mg/kg of captopril were insufficient to alter the activation threshold of TRPV4 and cause bronchoconstriction. Thus, although TRPA1 and TRPV4 are co-expressed with B_2 receptor in primary afferent fibres [44], the modulation of TRPV4 in the airways appears to be different from that of TRPA1, and higher bradykinin levels may be required to promote TRPV4 activation and induce bronchoconstriction. Furthermore, unlike TRPA1 and TRPV1, the function of TRPV4 in peripheral sensory neurons in the lungs has not been well explored and the activation pathways of this channel seem to be different from those of the other TRPs [46].

In order to continue exploring the role of TRPA1 and TRPV4 in the adverse airway effects induced by ACE inhibition, we demonstrated that antagonism of these channels may offer benefits in controlling the bronchoconstrictive responses induced by bradykinin in captopril-pretreated rats. Our data show that the bronchoconstriction induced by bradykinin in captopril-pretreated rats was markedly diminished by the TRPA1 antagonist. Thus, this result suggested again that a possible reduction in bradykinin breakdown, after treatment with captopril, could modulate this channel in a manner dependent on activation of B_2 receptor. In accordance with this hypothesis, Grace and collaborators showed that TRPA1 antagonism inhibits the tussive response to bradykinin in guinea pigs [8]. Indeed, the bronchoconstriction induced by allyl isothiocyanate in captopril-pretreated rats was inhibited by HOE140 pretreatment. Thus, these findings strongly suggest a relationship between B_2

receptor and TRPA1 channels in the adverse effects induced by captopril in rat airways. Interestingly, in captopril-pretreated rats the TRPV4 antagonist also inhibited the increased bronchoconstriction induced by bradykinin. Moreover, bronchoconstrictive responses induced by GSK1016790A in rats pretreated with captopril at the higher dose (5 mg/kg) were also inhibited by HOE140, suggesting an interaction between bradykinin and TRPV4.

On the other hand, we observed that the bronchoconstrictive responses induced by bradykinin in captopril-pretreated rats were not potentiated by the combination of TRPA1 and TRPV4 antagonists. Whereas bronchoconstriction was partially mediated by either TRPA1 or TRPV4, that the combined administration of TRPV4 and TRPA1 antagonists failed to show increased potency may indicate a possible role for other channels. Recently, De Oliveira et al. (2016) reported that TRPV1 could also be involved in the effects of captopril in the airways of rats [2]. Thus, the resistant component following the combination of TRPA1 and TRPV4 antagonists remains to be further elucidated.

Plasma extravasation is another important effect induced by ACEis treatment and characterized by increased microvascular permeability. Reinforcing the role of TRPA1 and TRPV4 in the airway alterations of rats, the plasma extravasation induced by captopril in the tracheae of animals was inhibited by pretreatment with HC030031 and HC067047.

Collectively, the data presented here support the hypothesis that TRPA1 and TRPV4, via a B_2 receptor activation-dependent mechanism, are involved as common effectors in the plasma extravasation and bronchoconstriction induced by captopril,

making them possible pharmacological targets to prevent or remediate ACEi-induced adverse respiratory reactions.

Disclosure

The authors have no conflicts of interest to declare.

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3.2 MANUSCRITO ORIGINAL 2

A ser submetido.

TRPV1 CHANNEL IS ESSENTIAL FOR AIRWAY INFLAMMATION AND HYPERRESPONSIVENESS INDUCED BY CAPTOPRIL

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ABSTRACT

Captopril administration results in adverse effects in airways as plasma extravasation, cough, airways hyperreactivity and interstitial lung infiltrates. The aim of the present study was to investigate the role of transient receptor potential vanilloid 1 (TRPV1) underlying adverse effects of different treatment regimens with captopril. Experiments were conducted to analyze the exacerbation of bronchoconstrictive and inflammatory responses triggered by the oral captopril administration (100 mg/kg) in acute versus sub-chronic regimens in rats, using selective agonist and antagonists of TRPV1 and bradykinin B_2 receptors and the ablation of TRPV1-expressing sensory neurons.

We have found that the hyperresponsiveness to capsaicin and bradykinin in captopril-treated rats seems to be an acute effect. The intravenous pre-treatment with HOE140, a B_2 receptors antagonist, decrease the hyperresponsiveness to bradykinin (75 ± 5% of inhibition) and abolished the exacerbation induced by capsaicin. Ablation of TRPV1-expressing sensory neurons also diminished the bronchial hyperresponsiveness to bradykinin (46 ± 5% of inhibition). In addition, the captopril-treatment regimens, acute (in a manner dependent of time; 6 hours after the administration) and sub-chronic during 10 days (1 hour after the administration, on tenth day), promoted a recruiting of inflammatory cells to the lung, accompanied by an increased total leukocyte count in bronchoalveolar lavage fluid (BALF) and bronchus-associated lymphoid tissue (BALT) hyperplasia, events reduced by the degeneration of TRPV1-positive sensory neurons. Furthermore, the treatment with captopril during 10 consecutive days increased the immunoreactivity of the TRPV1 channels in lung, an effect also reduced by ablation of TRPV1-expressing sensory neurons.

In conclusion, our data demonstrate that TRPV1 acts as an important mediator of captopril inflammatory responses in rat airways. From a therapeutic perspective, it can be interesting to manage patients suffering with cough, hyperresponsiveness and angioedema induced by angiotensin-converting enzyme inhibitor (ACEI).

Keywords: airway inflammation, hyperresponsiveness, TRPV1, captopril, bradykinin

Abbreviations: ACEIs: angiotensin-converting enzyme inhibitors; ANOVA: analysis of variance; B_2 receptors: bradykinin type 2 receptors; BALF: bronchoalveolar lavage fluid; BALT: bronchus-associated lymphoid tissue; DMSO: dimethyl sulfoxide; FDA: Food and Drug Administration; H&E: hematoxylin and eosin; i.p.: intraperitoneal injection; i.v.: intravenous injection; NaCl: Sodium chloride; PBS: phosphate buffered saline; s.e.m.: standard error of the mean; TRPs: transient potential receptors; TRPV1: transient potential receptors vanilloid 1; Veh: vehicle; v.o.: oral via;

1. Introduction

Unpredictable adverse effects are common reasons for low adherence to the antihypertensive therapy with angiotensin-converting enzyme inhibitors (ACEIs) (Israili and Dallas Hall, 1992; Vasekar and Craig, 2012). Airways adverse effects as angioedema, cough, bronchial abnormal response, lung infiltrates and bronchial hyperreactivity have been reported in the literature since 1985, after the first ACEI available, captopril, gained FDA approval (1981) (Sesoko and Kaneko, 1985; Schatz et al., 1989; Watanabe et al., 1996). Unfortunately, the prevention and resolution of these adverse effects on the airways are limited and difficult-to-treat. To date, there is no definitive and approved pharmacological treatment (Vasekar and Craig, 2012;

Overlack, 1996). Thus, understanding the precise pathophysiology of captopril-adverse effects in airways is important to achieve the management of patients.

A link with bradykinin levels has been described as a key to explain the adverse airways effects after ACEI treatment (Nussberger, 1998). Studies showed that the sensitization-mediate by bradykinin increased the number of coughs caused by ACEI in guinea pigs (Fox et al., 1996). This pro-inflammatory mediator activated bradykinin receptors type 2 (B_2 receptors) and sensitizes type C sensory fibers, where are co-expressed with transient potential receptors (TRPs) (Fox et al., 1996; Pethö and Reeh, 2012; Veldhuis et al., 2015). In fact, the lung is densely innervated by sensory afferent fibers, which express (the most) the transient receptor potential channel vanilloid member 1 (TRPV1), although TRPV1 is also expressed in non-neuronal cells (Grace et al., 2014). When stimulated by chemical, mechanical and thermal stimuli (Brederson et al., 2013), TRPV1-airways promote immune and inflammatory responses, via neurogenic and non-neurogenic mechanisms (Parenti et al., 2016). Thus, TRPV1 channels play an essential protective role in airway reflexes and also contribute to diseases (Grace et al., 2014; Canning et al., 2006).

Taking into account, the purpose of our study was to investigate the inflammatory responses induced by the different treatment regimens with captopril in rat airways and the contribution of TRPV1 channels in these responses. We hypothesized that TRPV1 play a major role in hyperresponsiveness and inflammation of airways induced by captopril, associated with a TRPV1 up-expression in lung.

2. Materials and methods

2.1 Animals

Male Wistar rats (250-300 g) were kept under controlled conditions of temperature (22 ± 2 ºC) and lighting (12 h light / dark cycles), with free access to water and food. For a period of at least 1 hour before the beginning the experiments, the feed was removed and the animals were kept in the laboratory for an adaptation period. All experimental procedures were carried out after approval by the Institutional Animal Care and Use Committee of the Federal University of Paraná (process number 800) and were in compliance with the ARRIVE guidelines, previously reported by Kilkenny and collaborators (Kilkenny et al., 2013). The *n* number of animals used in this study was the minimum necessary to obtain consistent data.

2.2 Airway resistance measurement

The animals were previously anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg) injected intraperitoneally (i.p.). After, a surgical procedure was performed to insert a cannula into cervical portion of trachea of rats, fixed with a suture wire and connected to artificial ventilation with room air (Ugo Basile Rodent Ventilator model 7025; 50 strokes/min; 10 ml kg^{-1} of room air; Ugo Basile, Comerico-Varese, Italy). The airway resistance was measure by a bronchospasm transducer (Ugo Basile Bronchospasm Transducer 17020, Ugo Basile, Italy). The method is based on the researchers Konzett and Rossler (1940) and Amdur and Mead (1958). The transducer was connected to a data acquisition system, the DataCapsule-Evo Digital Recorder (17308, Ugo Basile, Italy), which was connected to a computer for recording and analysis using the software LabScribe 3 TM. In all experiments the anesthetized animals were submitted for a stabilization period of at least 10 minutes

before the treatments. The results were expressed as an increase in airway resistance (volume-displacement H_2O/ml) above baseline value measured before administration of drugs (Dusser et al., 1988).

2.2.1 Hyperresponsiveness

To evaluate if ACEI could increase the sensitivity to agonists of B_2 receptor and TRPV1 channel in doses that *per se* not promoted bronchoconstriction, different groups of animals are treated with captopril 100 mg/kg (an antihypertensive dose effective in spontaneously hypertensive rats) administered by an oropharyngeal cannula (Antonaccio et al., 1979). The rats were randomly distributed in cages according to the following oral treatment regimens: Group vehicle, treated with saline/kg/day; Acute group, treated with a single dose of captopril (100 mg/kg), performing the experiments 1 hour after the administration; Group 9 days, treated with captopril (100 mg/kg/day) for 9 consecutive days, with experiments performed 24 hours after the last administration; and Group 10 days with captopril treatment (100 mg/kg/day) performed over 10 consecutive days, with experiments performed 1 hour after the last administration.

After the different captopril regimens treatment, the animals were anesthetized, cannulated and their respiratory rates are stabilized during 10 minutes. The B_2 receptors agonist bradykinin (0.03 µmol/kg) and the TRPV1 agonist capsaicin (0.03 µmol/kg) were injected via intravenous (i.v.) in doses unable to alter the airway resistance in vehicle treated animals.

In another series of experiments, animals treated acutely with captopril (100 mg/kg), 1 hour later, were anesthetized, cannulated and pretreated with HOE140 (0.01 µmol/kg; i.v.). Fifteen minutes later bradykinin (0.03 µmol/kg; i.v.) or capsaicin (0.03

μmol/kg; i.v.) were administered. Furthermore, the bronchial hyperresponsiveness induced by acute treatment with captopril and challenge with a low-dose of bradykinin was also evaluated in adult animals, with 8-10 weeks, previously desensitized with capsaicin. The degeneration of TRPV1-positive sensory neurons was performed in rats with 48 hours of life, injecting subcutaneously capsaicin (50 mg/kg) as previously described by Jancsó and collaborators (Jancsó et al., 1977). When given to newborn rats, capsaicin degenerate the neurons, identifying capsaicin-sensitive neuronal pathways (Jancsó et al., 1977). To confirm the success of the procedure the number of eye-wiping movements induced by ocular application of a capsaicin solution (0.1%) was counted in adult animals (Hoffmeister et al., 2014).

2.3 Inflammatory responses

2.3.1 Bronchoalveolar lavage and total leukocyte count

In order to investigate whether the different captopril treatment regimens cause pulmonary inflammation, the bronchoalveolar lavage fluid (BALF) of animals were collected to follow analyses. In rats treated in an acute manner (single-dose protocol) with captopril (100 mg/kg; v.o.), the BALF was collected in two time-points, 1 hour and 6 hours after the administration (Costa et al., 2002). The BALF of sub-chronic groups, treated during 9 or 10 days, was collected on the tenth day, 24 hours or 1 hour after the last administration, respectively.

The BALF was collected of euthanized animals (overdose of thiopental 100 mg/kg, i.p.). The trachea was exposed and cannulated with a polyethylene tube, connected to a syringe. The lungs were washed by flushing with phosphate buffered saline (PBS) solution. The PBS buffer was instilled through the tracheal cannula as one 1

ml and was aspirated again; the procedure was performed 3 times in order to obtain the exudate. The fluid recovered after each aliquot instillation was combined and centrifuged (290 g for 8 min at 4ºC). The cell pellet was resuspended in 100 µl of PBS buffer and the total cell counts was determined using a Neubauer chamber (American Optical, Southbridge, MA) after the addition of Türk's solution (0.5 mg/ml in PBS) in optical microscope (400 ×). The results were expressed by total number of cells (× 10^7). The total leukocyte count was also investigated in group of animals with degeneration of sensory neurons that express the TRPV1 channels by the capsaicin pretreatment during neonatal period (Deg group). The procedure was previously described by van Hoecke and collaborators, with some modifications (van Hoecke et al., 2017).

2.3.2 Histological Analysis

To perform the histological analysis, animals were euthanized with an overdose of thiopental (100 mg/kg, i.p.) and the left lobe of lung (with only one lobe and a simple structure) was collected from rats submitted to the different therapeutic regimens with captopril. The tissues were fixed in ALFAC solution (alcohol 80%, formaldehyde 40% and glacial acetic acid) and embedded in paraffin. Paraffin blocks were sectioned into 5 µm thick sections and sequentially staining with hematoxylin and eosin (H&E). The extent of lung inflammation was evaluated in the H&E-stained lung by a pathologist who was blind to the experimental grouping (Suda et al., 1999).

2.4 Immunohistochemistry analysis

Immunohistochemistry for TRPV1 channels was performed on the left lung of rats submitted to different captopril treatment regimens. After a transcardiac perfusion

procedure with saline (0.9% NaCl), to remove the lung, the tissues were fixed in ALFAC solution (alcohol 80%, formaldehyde 40% and glacial acetic acid) and embedded in paraffin. Lung sections of paraffin blocks were sectioned (5 µm thickness), and after blocking reactions, were incubated overnight with primary antibody against TRPV1 (1:100, Alomone Labs) at 4 °C. Subsequently, the sections were washed for 4 times with 1% BSA/PBS and incubated with peroxidase conjugated secondary antibody (goat anti-rabbit, 1:100, Santa Cruz Biotechnology) at room temperature in a humid chamber for 1 hour. Positive signals were developed using chromo-gen diaminobenzidine (DAB Substrate Kit, BD Pharmingen™). After, the sections were washed three times with PBS, dehydrated and counter stained with H&E. Immunoreactivity for TRPV1 was visualized under a Zeiss Axio Imager Z2 with the capture software Metafer 4/VSlide. The images were analyzed with Image-Pro Plus 6.0 software (Maria-Ferreira et al., 2018).

2.5 Drugs and reagents

The most drugs and reagents were purchased from Sigma Chemical Co., St. Louis, USA except the rabbit anti-TRPV1 antibody poly clonal (Alamone Labs), the goat anti-rabbit secondary antibody (Santa Cruz Biotechnology), the DAB Substrate Kit (BD Pharmingen™) and the captopril (MULTILAB Indústria e Comércio de Produtos Farmac LTDA.). The solutions were diluted on the day of the experiment just before use. The final concentrations of dimethyl sulfoxide (DMSO) and Tween 80 did not exceed 0.1% in solutions. Only capsaicin dilution for degeneration- neonatal pretreatment was made in 10% ethanol and 10% Tween 80 in 0.9% NaCl solution.

2.6 Statistical analysis

The results are expressed as the mean + standard error of mean (s.e.m.). The statistical significance between the groups was assessed by the one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test or Bonferroni test. The unpaired Student's *t*- test also was applied when appropriate. GraphPad Prism software version 5.01 and 6.01 were used. In all experiments values of $P \leq 0.05$ were considered to be statistically significant.

3. Results

3.1 Captopril sensitizes acutely the airways by a mechanism involving TRPV1-positive sensory neurons and B_2 receptors

The acute oral administration of captopril (100 mg/kg) promoted bronchoconstriction in rats after challenge with agonist bradykinin 0.03 μmol/kg (1.57 ± 0.09 volume-displacement H_2O/ml) or capsaicin 0.03 μmol/kg (0.72 ± 0.14 volume-displacement H_2O/ml) in doses that *per se* not induce bronchoconstrictive responses (Figure 1A, B).

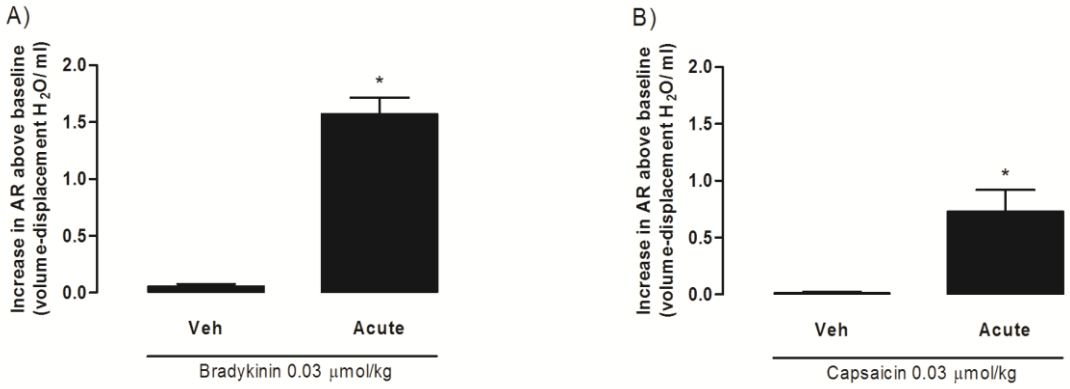

Figure 1. Effect of acute captopril administration in rat airways. Airway hyperresponsiveness to bradykinin (A) and capsaicin (B) induced by captopril acute treatment (100 mg/kg; v.o.). In acute group the experiments were performed 1 hour after the last administration of the ACEI. Each point represents the mean (S.E.M.) of seven rats. The asterisks denote significance levels: *P ≤ 0.05 compared to vehicle treated group (Veh). T-Test.

To confirm the bradykinin role in airways captopril-sensitization, we demonstrated that the pre-treatment intravenous with HOE140 (0.01 µmol/kg), a B_2 receptors antagonist, decrease the hyperresponsiveness to bradykinin (0.03 µmol/kg; 75 ± 5% of inhibition) in comparison of saline pretreated group (Figure 2A) and abolished the exacerbation induced by capsaicin (0.03 µmol/kg; Figure 2B) in captopril-acute treated rats (100 mg/kg). We also examined the effect of TRPV1-positive sensory neuron's degeneration on hyperresponsiveness to bradykinin induced by captopril. The capsaicin pre-treatment during neonatal period (Deg group) diminished the bronchial hyperresponsiveness to bradykinin (0.03 µmol/kg; 46 ± 5% of inhibition) in adult rats treated in an acute manner with captopril (100 mg/kg) (Figure 2C).

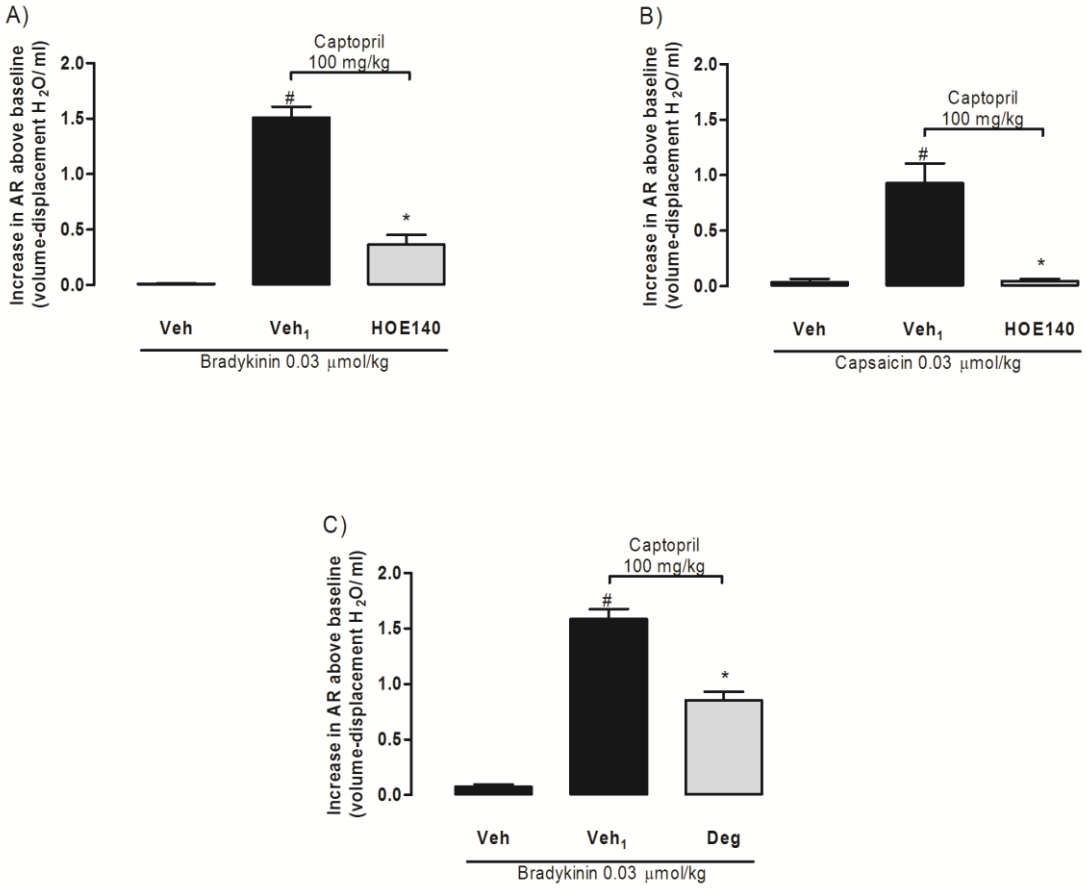

Figure 2. Role of B_2 receptors and TRPV1-positive sensory neurons in hyperresponsiveness induced by acute captopril treatment. Intravenous pre-treatment with B_2 receptors antagonist HOE140 (0.01 μmol/kg; A, B) and the degeneration of TRPV1-sensory neurons by capsaicin neonatal pre-treatment (50 mg/kg s.c.; Deg group; C) reduces the airways hyperresponsiveness to bradykinin and capsaicin induced by acute captopril treatment (100 mg/kg; v.o.). Experiments were performed 1 hour after the administration. Each point represents the mean (S.E.M.) of seven rats. *P ≤ 0.05 compared to vehicle pretreated group (Veh_1). #P ≤ 0.05 compared to vehicle treated group (Veh). Two-way ANOVA followed by Newman-Keuls post-hoc test.

Similar results were observed in rats treated sub-chronically during 10 days and challenged with the agonists bradykinin (0.03 μmol/kg; 1.32 ± 0.08 volume-displacement H_2O/ml) and capsaicin (0.03 μmol/kg; 0.77 ± 0.09 volume-displacement H_2O/ml) 1 hour after the last captopril administration on tenth day (Figure 3A, B). However, in groups of animals treated during 9 consecutive days with captopril and

challenged with the agonists, 24 hours after the last administration of the ACEI, the sensitization of airways was absent.

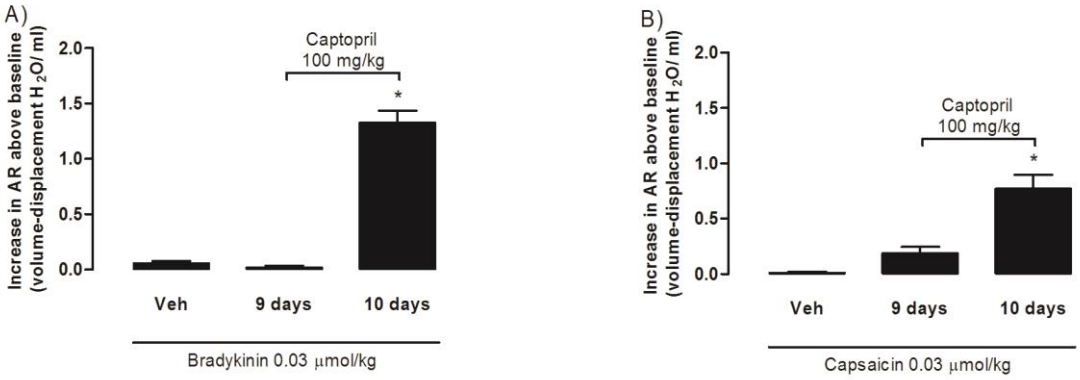

Figure 3. Effect of sub-chronic captopril administration in rat airways. Airways hyperresponsiveness to bradykinin (A) and capsaicin (B) induced by sub-chronic regimens of treatment with captopril (100 mg/kg; v.o.). In group treated during 9 consecutive days the experiments were performed on the tenth day, 24 hours after the last treatment. In group treated during 10 consecutive days the experiments were performed one hour after the last treatment Each point represents the mean (S.E.M.) of seven rats. *P ≤ 0.05 compared to the others groups. Two-way ANOVA followed by Newman-Keuls post-hoc test.

3.2 Captopril induce inflammatory responses in airways

We evaluated the total leukocytes count in BALF of animals treated with captopril (100 mg/kg) in the different therapeutic regimens. In acute treated group the content of total leukocytes in BALF was greatly elevated in a manner time-dependent, only 6 hours after the administration (1.19×10^7 cells), when compared to 1 hour captopril-treated point (0.19×10^7 cells) or vehicle treated group (Veh, 0.32×10^7 cells) (Figure 4A). Corroborating, the histopathology analysis of the H&E-stained sections of lungs of rats showed an increase in size of BALT, also in a manner dependent of time, only when tissues were collected 6 hours after the acute treatment with ACEI (Figure 4D),

when compared to 1 hour captopril-treated group (Figure 4E) or vehicle-treated group (Figure 4C).

In sub-chronic 10 days group, the total leukocyte count in BALF also increased (2.02 × 10^7 cells) when compared to vehicle-treated group (Veh, 0.37 × 10^7 cells; Figure 5A). Differently of observed in acute group, the cell increase in BALF could be observed in the first hour after the last treatment, on tenth day. However, in the group treated during 9 consecutive days, with BALF collected 24 hours after the last administration of the ACEI, we observed that the total leukocyte count regressed significantly (0.46 × 10^7 cells) in comparison with sub-chronic 10 days group and was not significantly different of vehicle-treated group (Figure 5A). Reinforcing these findings, the H&E-stained sections of lungs demonstrated that in 10 days group occurred a significant BALT hyperplasia near the bronchiole walls (Figure 5D), in comparison of 9 days treated-group (Figure 5E), which not showed significant histologic alterations, or vehicle-treated group (Figure 5C).

3.3 Degeneration of TRPV1-positive neurons reduces the inflammatory responses induced by captopril

We also evaluate the participation of sensitive capsaicin neurons in the inflammatory responses induced by the different regimens with captopril (100 mg/kg). The ablation with capsaicin of sensory neurons that express TRPV1 inhibited significantly (Deg group, 0.2 × 10^7 cells) the increase in total leukocyte count in BALF of acute captopril treated rats (1.19 × 10^7 cells; BALF collected 6 hours after the ACEI administration) (Figure 4F). Indeed, we also observed that the total leukocyte count in BALF of sub-chronic 10 days group (1.8 × 10^7 cells), with BALF collected 1 hour after the captopril

administration, on tenth day, was also inhibited by the capsaicin pre-treatment (Deg group, 0.1 × 10^7 cells; Figure 5F).

In the same direction of BALF results, the histological evaluations of lungs showed that the ablation of TRPV1-positive sensory neurons prevent the increase of BALT size promoted by acute administration of captopril (tissues collected 6 hours after the treatment; Figure 4D) and reduced significantly the BALT hyperplasia induced by repetitive administration of the ACEI during 10 days (Figure 5D).

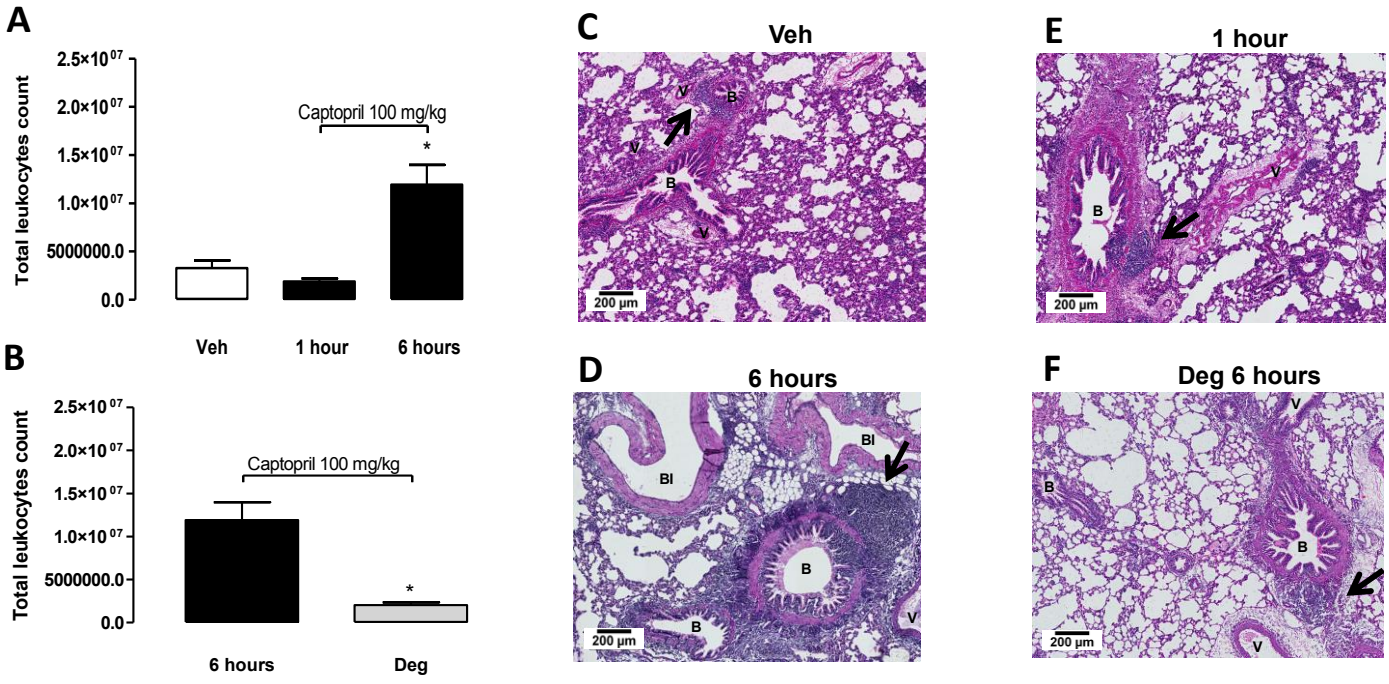

Figure 4. Histological sections of lung of acute captopril treated groups. Representative photomicrographs of rat lung tissues in H&E-stained sections (C-F). Captopril acute treatment (100 mg/kg; v.o.), in a manner dependent of time, induces BALT increasing (D) and rise the total leukocytes count in BALF (A). These effects were significantly reduced by the capsaicin neonatal pre-treatment (50 mg/kg s.c.; Deg group) (F and B). Each point represents the mean (S.E.M.) of seven rats. The asterisks denote significance levels: *P ≤ 0.05 compared to the other groups. #P ≤ 0.05 compared to the 6 hours group. Two-way ANOVA followed by Newman-Keuls post-hoc test. V= pulmonary vasculature; B= bronchiole; BI= intrapulmonar bronchi; *= BALT.

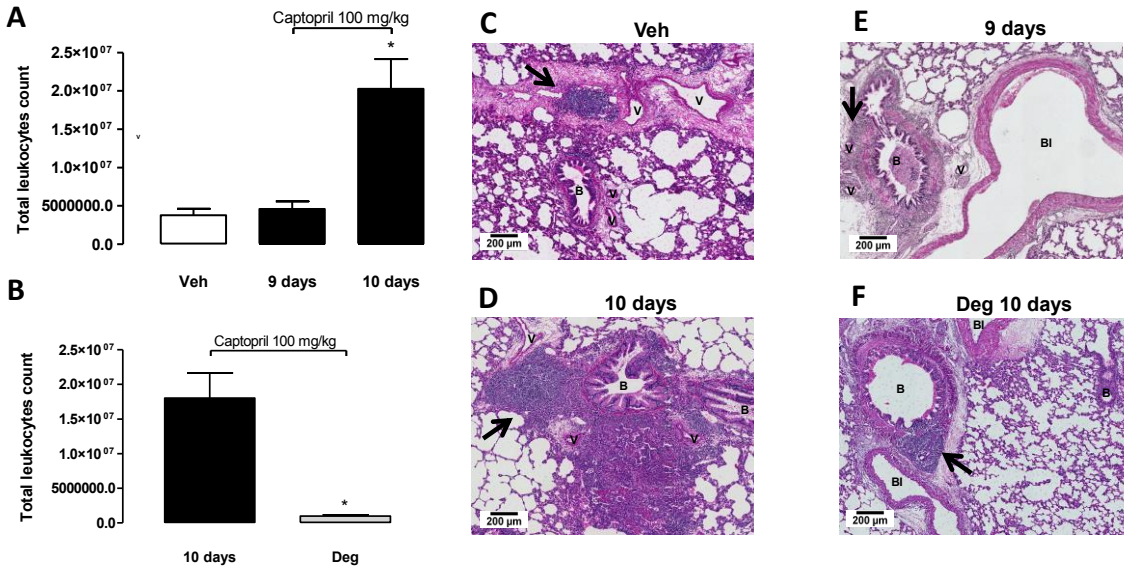

Figure 5. Histological sections of lung of sub-chronic captopril treated groups. Representative lung photomicrographs of rat lung tissues in H&E-stained sections (C-F). Captopril treatment during 10 consecutive days (100 mg/kg), 1 hour after the administration, on tenth day, increased the total leukocytes count in BALF (A) and BALT hyperplasia (D) when compared to the others groups. These effects were significantly reduced by the capsaicin neonatal pre-treatment (50 mg/kg s.c.; Deg group) (B, F). In 9 days treated group, with tissue and BALF collected 24 hours after the last administration, on tenth day, the inflammatory parameters regressed significantly (A, E). Each point represents the mean (S.E.M.) of seven rats. The asterisks denote significance levels: *P ≤ 0.05 compare to the other groups. #P ≤ 0.05 compared to the group treated during 10 consecutive days with captopril. Two-way ANOVA followed by Newman-Keuls post-hoc test. V= pulmonary vasculature; B= bronchiole; BI= intrapulmonar bronchi; *= BALT.

3.4 Effect of captopril on TRPV1-immunoreactivity in the rat lung

We also performed an immunohistochemical analysis to determine the expression profile of TRPV1 channels in rat lung. The results showed an intense TRPV1 immunoreactivity in lung of captopril sub-chronic treated group after 10 consecutive days of treatment (100 mg/kg; Fig 7C), with no alterations of TRPV1 patterns in the others groups evaluated (Fig 6A – C and 7A, B). The effect was demonstrated by the increases in TRPV1 immunostaining scores compared to vehicle-treated and other captopril regimens (Fig 6E and 7E). Moreover, according to the others inflammatory parameters evaluated, the degeneration of TRPV1-positive sensory neurons also

reduced the increased TRPV1-immunoreactivity of captopril sub-chronic 10 day's group (Fig 7D, E).

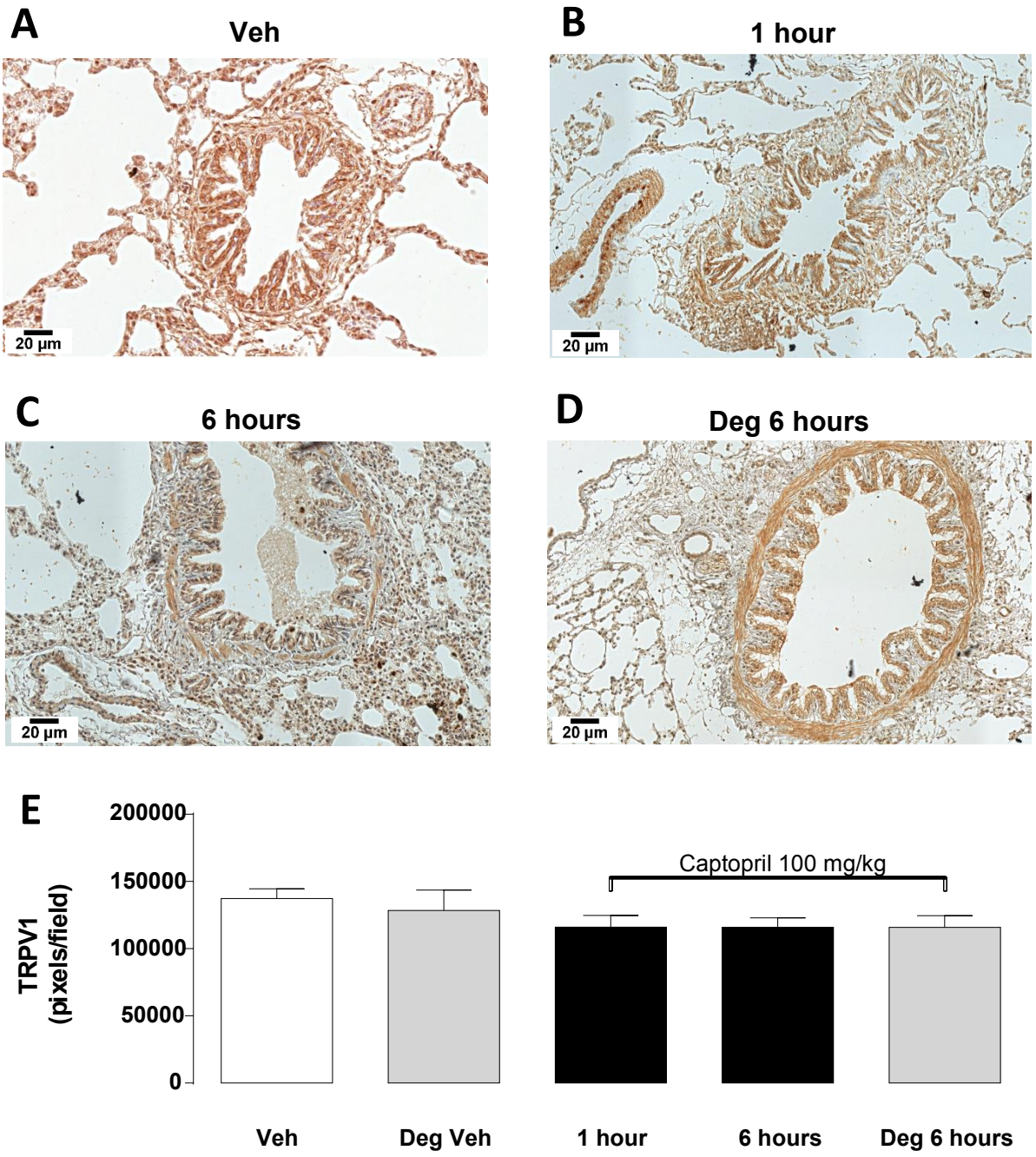

Figure 6. Immunoreactivity for TRPV1 in lung sections of rats treated acutely with captopril. Immunohistochemical staining for TRPV1 from lung sections of vehicle (A) and captopril acute treated groups, with left lung collected 1 hour (B) or 6 hours (C) after the ACEI administration. Immunostaining scores for TRPV1 (E). Deg group represents the animals pretreated with capsaicin during neonatal period (50 mg/kg s.c.; D, E) with assay performed 8 or 10 weeks after, in adult animals. Each point represents the mean (S.E.M.) of three rats. Two-way ANOVA followed by Bonferroni post-hoc test.

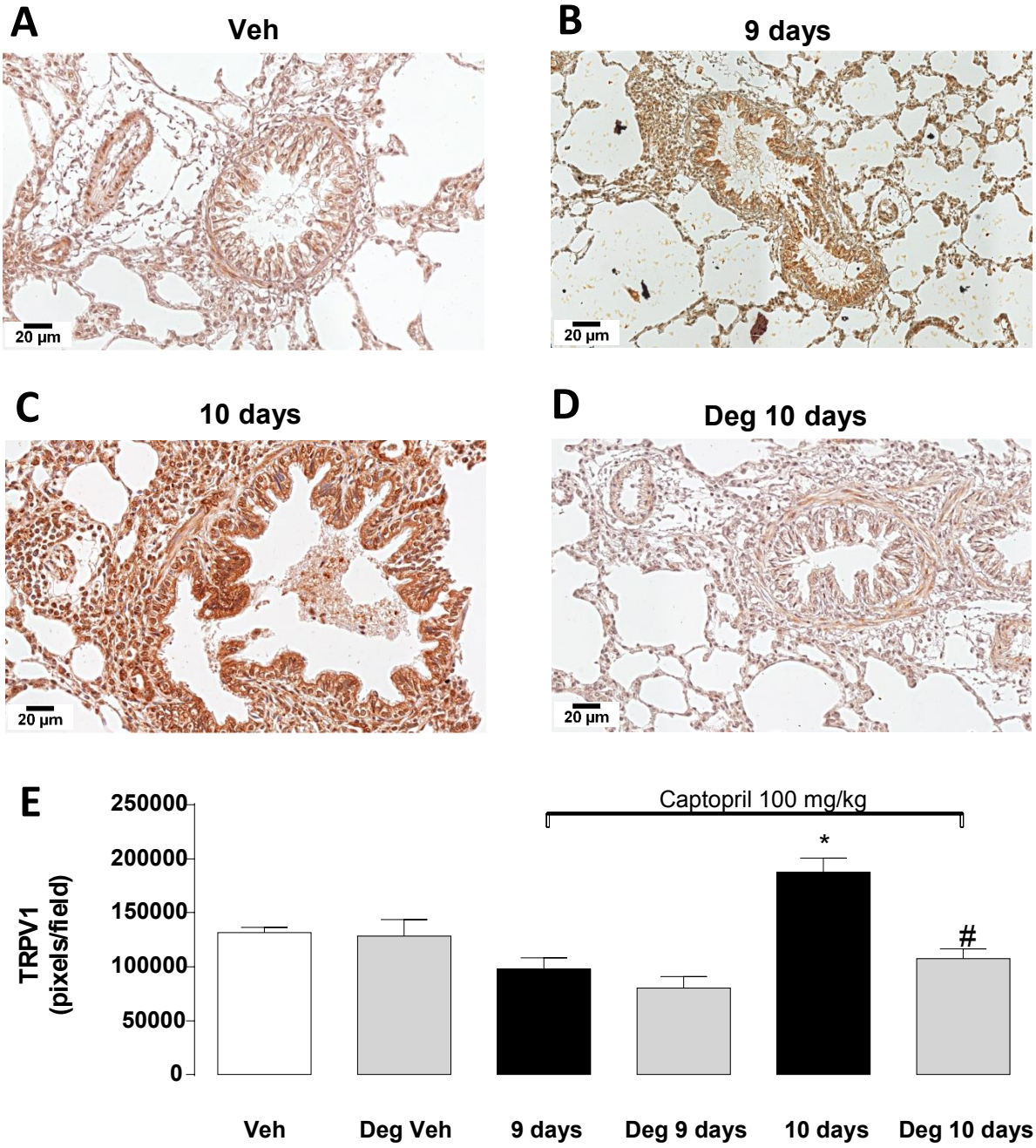

Figure 7. Immunoreactivity for TRPV1 in lung sections of rats treated in a sub-chronic regimen with captopril. Immunohistochemical staining for TRPV1 from lung sections of vehicle (A) and captopril sub-chronically treated groups (100 mg/kg); 9 days with tissues collected on tenth day, 24 hours after the last administration (B) or 10 consecutive days, with tissues collected 1 hour after the last administration (C). Immunostaining scores for TRPV1 (E). Deg group represents the animals pretreated with capsaicin during neonatal period (50 mg/kg s.c.; D, E), with assay performed 8 or 10 weeks after, in adult animals. Each point represents the mean (S.E.M.) of three rats. The asterisks denote significance levels: *P ≤ 0.05 compared to the others groups treated with vehicle or captopril. #P ≤ 0.05 compared to the group treated

during 10 consecutive days with captopril. Two-way ANOVA followed by Bonferroni post-hoc test.

4. Discussion

The data presented here support the idea that captopril-sensitization of TRPV1 can induce neurogenic inflammation give rise bronchial hyperresponsiveness and airway inflammation, hallmarks of respiratory diseases. We observed a sensitization of airways to bradykinin and capsaicin in animals treated in acute and sub-chronic regimen with captopril. This effect seem occurs independently of regimen of treatment applied and is underlined by the observation that a single dose of captopril can to induce potentiating of bronchoconstrictive response to bradykinin or capsaicin, although this effect is not evidenced 24 hours after drug discontinuation even when it was administrated during 9 consecutive days. Thus, these findings suggested that the endogenous bradykinin levels, maintaining by ACE inhibition, seems to be a crucial factor for transiently sensitize and enhance the airway resistance of animals treated with captopril.

In clinical, cough and angioedema by ACEIs treatment may develop within hours or at most weeks after the first dose, although cases after long-term therapy was also reported (Israili and Dallas Hall, 1992). Oike and collaborators, for example, described a fatal angioedema and leukocytosis within the first few doses of enalapril (Oike et al., 1993). Morice and others also showed that 2 hours after 25 mg of captopril by mouth shift the sensitivity of capsaicin inhaled in airways of normal subjects (Morice et al., 1987). Therefore, the occurrence of airways adverse effects induced by ACEIs is unpredictable and was confirmed by studies showing that the symptoms in clinical seems to improve substantially within hours after stopping the drug (Israili and Dallas Hall, 1992; Banerji et al., 2016). For this reason, the

discontinuation of therapy appears an immediate recommendation to prevent cough and angioedema occurrence in humans (Vasekar and Craig, 2012). Corroborating, we showed a complete resolution of bronchial sensitization 24 hours after suspension of captopril administration.

The exacerbation of bronchoconstrictive response observed during the ACEI treatment occurred by a mechanism sensitive to pharmacological blockaded of B_2 receptors and ablation of TRPV1-expressing sensory neurons. Indeed, several preclinical studies also have shown the possible TRPV1 and B_2 receptors involvement in the adverse effects induced by ACEIs in airways of different animal models. In 1996, Takahama and collaborators demonstrated that the acute and chronic treatment with captopril increase the cough induced by capsaicin in guinea pigs (Takahama et al., 1996). Data from our laboratory also demonstrated that the pretreatment with B_2 receptors antagonist or TRPV1 antagonist, as well as degeneration of TRPV1-expressing sensory neurons, inhibited the plasma extravasation induced by acute intravenous administration of captopril in artificially ventilated rats (de Oliveira et al., 2016). Additional study have generated evidence that captopril increases BK-induced bronchoconstriction in guinea pigs through the release of tachykinins from sensory neurons (Arakawa et al., 1996). Thus, the present data support that captopril, in a bradykinin dependent pathway; modulated the TRPV1-positive neurons triggering airways hyperresponsiveness.

It has already been proposed that the effects of bradykinin in airways is due its ability in active B_2 constitutive receptors and sensitize TRPV1 channels expressed in primary sensory neurons (Grace et al., 2014; Schulze-Topphoff et al., 2008; Belvisi and Birrel. 2017; Gouin et al., 2017). Once activated, these TRPV1-expressing sensory neurons release neuropeptides such as substance P from their peripheral

nerve endings, which modulate bronchial smooth muscle-tone, increased vascular permeability, promotes leukocyte adherence to the vascular endothelium and migration in airways (Medeiros et al., 2001; Katayama et al., 1993). Additionally, this neuronal stimulation seems to exert an immunomodulatory effect for development or function of BALT, part of the integrated mucosal immune system of rats (Auais et al., 2003). In humans, a related tissue (inducible BALT) can be occasionally found in the lungs upon inflammation or infection (Moyron-Quiroz et al., 2004). Here, our findings demonstrated that acute, in a manner dependent of time, and sub-chronic treatment regimens with captopril triggered an inflammatory process in the rat airways, characterized by an increase in total leukocytes count in BAL and BALT hyperplasia accompanied by sub-epithelial, peribronchial and perivascular infiltration of inflammatory cells.

In this sense, again, we noticed the fundamental role of bradykinin. This is supported by the finding showing that 24 hours after stopping captopril treatment significantly reduces the inflammatory parameters analyzed, while the continuous administration seems to favor the recruitment of inflammatory cells, promoting just 1 hour after captopril administration, on tenth day, a significant inflammatory response. Furthermore, the sensory neurons expressing TRPV1 also play an important role, once their ablation markedly reduce the inflammatory responses, supporting the causal relationship between bradykinin and the sensitization of capsaicin-sensitive C fibers in the mechanism by ACEIs promotes adverse effects in airways.

It is plausible to speculate that due to inflammatory process and sensitization of airways induced by ACEI treatment can promote an over-expression of TRPV1 channels. Several literatures postulated a relation between the exaggerated tussive response to capsaicin and increased expression of TRPV1 in airways epithelial

nerves or non-neuronal cells in patients suffering from chronic cough or asthma (Groneberg et al., 2004; Mitchell et al., 2005; McGarvey et al., 2014). Indeed, TRPV1 channels have been expressed in the highest level in sensory neurons of respiratory tract and a significant expression is also observed in non-neuronal cells, as bronchial epithelium, microvascular endothelial and others lung cell types (Grace et al., 2014; Groneberg et al., 2004). Here, our quantitative immunohistochemistry results revealed that prolonged treatment with captopril modify the cellular expression of TRPV1 on lung. We hypothesized that this TRPV1 up-regulation depends on maintenance of bradykinin amounts through daily administration of captopril, once 24 hours after the discontinuation of treatment the effect was markedly reduced. The ablation of capsaicin sensitive sensory neurons during neonatal period also prevented this TRPV1 over-expression. In this context, we suggested that increased levels of bradykinin by ACE inhibition can, besides reduction of TRPV1-activation threshold, be also related to the channel up-regulation in rat lung. Recently, Mistry and collaborators previously demonstrated this possibility in vitro model; of bradykinin promoting an up-regulation of TRPV1 mRNA expression in a culture of nociceptive primary sensory neurons after 2 days of exposition (Mistry et al., 2014).

Although the mechanism underlying captopril promotes airway adverse effects continue uncertain, the data presented confirmed the central role of bradykinin and TRPV1 receptors. In conclusion, we proposed that the suppression or reversing sensitization of TRPV1 channels, promoted by raised level of bradykinin by ACE inhibition, is an important pathway for modulate the captopril-inflammatory airways effects. In clinical, so far, the treatment for angioedema, cough or pulmonary inflammation induced by this class of drugs consists in the interruption of drug administration, exposing the patients to the risk of their current situation (Vasekar

and Craig, 2012; Dicpinigaitis, 2006). In this sense, the presented data indicate that the TRPV1 channels modulation could be a useful local strategy for resolution of airway symptoms induced by ACEIs.

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Disclosure statement

The authors have declared no conflicts of interested.

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4 CONCLUSÃO FINAL

Efeitos adversos após o tratamento com IECAs como tosse (mais comum) e angioedema (associado a uma considerável morbidade) são razões comuns de descontinuação e baixa adesão à terapia (WAKEFIELD; THEAKER; PEMBERTON, 2008; VASEKAR; CRAIG, 2012). Até o momento não há uma intervenção farmacológica definitiva e aprovada para o manejo desses efeitos adversos, uma vez que seus mecanismos patofisiológicos não foram totalmente elucidados. No entanto, a potencialização da sinalização da bradicinina, alcançada pela inibição da ECA, sugere a participação desse mediador inflamatório (ACHARYA et al., 2003). Estudos demonstraram que os canais TRPV1, TRPA1 e TRPV4 podem ser sensibilizados por bradicinina em modelos experimentais de tosse e hiperalgesia

(GRACE et al., 2012; BENEMEI et al., 2015; COSTA et al., 2018). Esses receptores estão amplamente expressos no trato respiratório e atuam como transdutores de estímulos químicos nocivos, orquestrando respostas inflamatórias de modo a manter a função e a homeostasia pulmonar (BELVISI; BIRRELL, 2017). Neste estudo, apresentamos o papel dos canais TRPV1, TRPA1 e TRPV4 nos efeitos adversos induzidos por captopril, um IECA, nas vias aéreas de ratos.

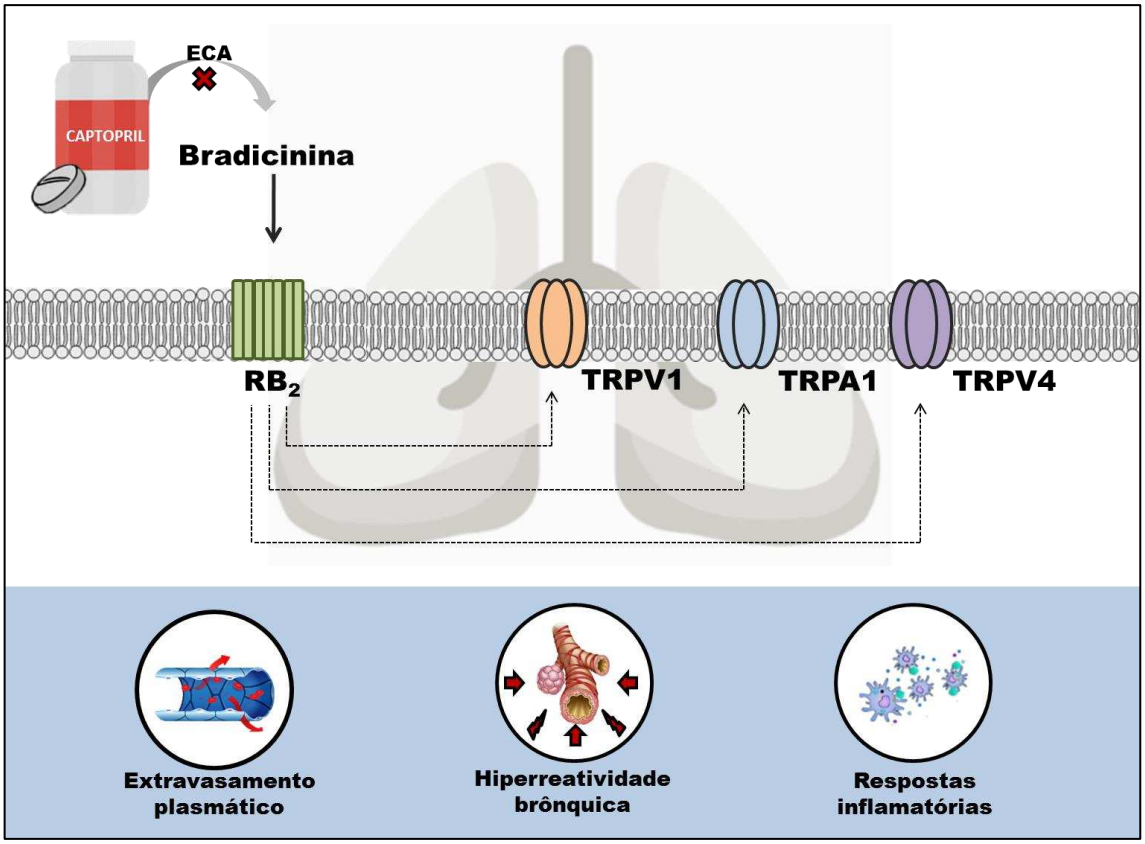
Inicialmente demonstramos que a administração aguda (via oral ou intravenosa) e sub-crônica (via oral) de captopril, por si só, não alterou a resistência das vias aéreas, corroborando com os dados publicados por Greenberg e colaboradores no ano de 1979 (GREENBERG et al., 1979). No entanto, que a inibição da ECA, ao reduzir a degradação dos níveis circulantes de bradicinina, favorece a ativação de seus receptores metabotrópicos B_2, sem evidências da participação de receptores B_1, promovendo sensibilização periférica dos canais TRPV1, TRPA1 e TRPV4. Os resultados demonstram que uma única administração do IECA, independentemente da via de administração escolhida, é capaz de diminuir seus limiares de ativação, fazendo com que a administração de agonistas desses canais em doses baixas promova a ativação de neurônios sensoriais onde estão co-expressos e gerem respostas do tipo neurogênicas como hiperreatividade brônquica, broncoconstrição e extravasamento plasmático.

Na clínica, os efeitos adversos induzidos por IECAs sobre as vias aéreas podem se manifestar dentro de horas e até mesmo semanas após a primeira dose (ISRAILI; DALLAS HALL, 1992). No presente estudo, nós demonstramos que a alteração da sensibilidade dos TRPs está diretamente relacionada aos níveis de bradicinina circulantes e a administração diária de captopril, uma vez que a descontinuação do tratamento após 24 horas melhorou substancialmente todas as respostas inflamatórias avaliadas. Também observamos que a administração oral de captopril por si só induziu um processo inflamatório nos pulmões de ratos, de modo tempo-dependente, caracterizado pelo aumento no número total de leucócitos no lavado broncoalveolar, infiltração de células inflamatórias e hiperplasia de tecido linfoide associado ao brônquio (parte do sistema imunológico integrado a mucosa de ratos). Demonstramos ainda que essas respostas inflamatórias podem ser prevenidas em animais previamente submetidos à ablação de neurônios sensoriais que expressam o canal TRPV1.

Com frequência, vias respiratórias hipersensíveis e inflamadas apresentam vias neurais sensoriais desreguladas. Assim, além da sensibilização, um segundo modo de modular a atividade dos canais TRPs seria a alteração da sua expressão clássica. Nesse sentido, demonstramos que o tratamento subcrônico com captopril durante 10 dias consecutivos foi capaz de aumentar a expressão celular do canal TRPV1 no pulmão de ratos. Assim como os demais efeitos descritos neste estudo, esta modulação ascendente do TRPV1 também parece ser diretamente dependente da administração diária de captopril, dos níveis circulantes de bradicinina e também envolve a participação de neurônios sensoriais que expressam o canal, visto que 24 horas após a descontinuação do tratamento sua expressão regride significativamente e que a ablação de neurônios sensoriais sensíveis a capsaicina preveniu o aumento da sua expressão. Logo, os dados sugerem um mecanismo adicional para modificação das condições inflamatórias induzidas por captopril nas vias aéreas.

Em conjunto, o trabalho realizado nesta tese demonstra os canais TRPV1, TRPA1 e TRPV4 como importantes mediadores das respostas inflamatórias pulmonares induzidas por captopril (FIGURA 6). Frente à escassez de um tratamento farmacológico padronizado para reversão da tosse e angioedema induzidos por IECAs, uma necessidade médica ainda não atendida, e sua recomendação como fármacos de primeira escolha para intervenção farmacológica inicial em pacientes hipertensos por *guidelines* recentes, o que tende a aumentar seu uso clínico e concomitantemente a incidência desses efeitos adversos, nossos resultados são relevantes ao sugerir que a modulação desses canais possa oferecer uma estratégia farmacológica atraente no gerenciamento e tomada de futuras decisões.

FIGURA 6 – HIPÓTESE PROPOSTA PARA AS RESPOSTAS INFLAMATÓRIAS INDUZIDAS POR CAPTOPRIL NAS VIAS AÉREAS DE RATOS



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