UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM FARMACOLOGIA

Gabriele Cheiran Pereira

DEPRESSÃO: ASPECTOS FISIOPATOLÓGICOS E O EFEITO TIPO-ANTIDEPRESSIVO DA APOCININA

Santa Maria, RS, Brasil
2020

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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Farmacologia, Área de Concentração em Neuropsicofarmacologia e Imunofarmacologia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestra em Farmacologia**.

Orientador: Prof. Dr. Guilherme Vargas Bochi
Coorientadora: Prof. Dra. Gabriela Trevisan

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This study was financied in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001

Pereira, Gabriele Cheiran
Depressão: aspectos fisiopatológicos e o efeito tipo antidepressivo da apocinina / Gabriele Cheiran Pereira.- 2020.
141 p.; 30 cm

Orientador: Guilherme Vargas Bochi
Coorientadora: Gabriela Trevisan
Dissertação (mestrado) - Universidade Federal de Santa Maria, Centro de Ciências da Saúde, Programa de Pós Graduação em Farmacologia, RS, 2020

1. Microglia 2. Eixo HPA 3. BDNF 4. Glicocorticoide 5. Antioxidante I. Vargas Bochi, Guilherme II. Trevisan, Gabriela III. Título.

sistema de geração automática de ficha catalográfica da UFSM. dados fornecidos pelo autor(a). sob supervisão da direção da divisão de processos técnicos da biblioteca central. bibliotecária responsável paula schoenfeldt patta crb 10/1728.

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Aprovado em 17 de fevereiro de 2020:

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Santa Maria, RS
2020

DEDICATÓRIA

Esse trabalho é dedicado à toda minha família, especialmente à minha avó paterna, Inês Maria Demétrio Pereira, que acompanhou desde os meus primeiros passos até esse momento importante e que agora nos acompanha de um lugar melhor.

AGRADECIMENTOS

Agradeço primeiramente à Deus, por ser bom e sábio o tempo todo;

Aos meus pais, Cleber e Magda, pelo amor, apoio e incentivo incondicional;

À minha irmã Rafaela, pelo apoio e ajuda na elaboração das figuras que compõem esse trabalho;

Ao meu noivo, Miguel Plentz Mestre, pelo amor, carinho, companheirismo, incentivo e compreensão;

Ao meu orientador, prof. Guilherme Vargas Bochi, pela orientação, disponibilidade, suporte, incentivo e amizade durante esses dois anos e meio de convivência. Agradeço também a oportunidade de integrar seu grupo de pesquisa e pela confiança em mim depositada para a realização desse trabalho e de tantos outros experimentos;

À minha co-orientadora, Gabriela Trevisan, pelo suporte científico, sugestões e discussões;

Aos nossos queridos IC's Jéssica, Elisa, Rossano, Laura, Luís Fernando e Luís Guilherme pela ajuda em todos os experimentos;

Às colegas do laboratório NeuronLab, especialmente à Amanda, Brenda e Camila pela convivência e troca de experiência;

Às minhas amigas e mães científicas Marina de Souza Vencato e Karen Luise dos Santos Moreira, por despertarem em mim o gosto pela pesquisa ainda no primeiro semestre da graduação;

À prof. Sara Marchesan de Oliveira e alunas, especialmente à Maria Fernanda, pela ajuda e experiência compartilhada;

Ao prof. Mauro Schneider Oliveira e alunas, pela disponibilidade e ensinamentos;

À Universidade Federal de Santa Maria e ao Programa de Pós-Graduação em Farmacologia, em especial aos professores e nossa prestativa secretária Zeli;

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pelo suporte financeiro;

E a todos que, de alguma maneira, contribuíram para a minha formação e para que a realização desse trabalho.

“Noventa por cento do sucesso baseia-se simplesmente em insistir.”

Woody Allen

RESUMO

DEPRESSÃO: ASPECTOS FISIOPATOLÓGICOS E O EFEITO TIPO-ANTIDEPRESSIVO DA APOCININA

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COORIENTADORA: Gabriela Trevisan

A depressão é considerada um desafio à saúde pública ao redor do mundo. Segundo a Organização Mundial da Saúde, mais de 300 milhões de pessoas (aproximadamente 6% da população mundial) são acometidas por essa desordem. Considerando que a depressão está intimamente relacionada à alta taxa de morte por suicídio e que grande parcela dos pacientes é refratária a qualquer um dos agentes antidepressivos disponíveis (entre 30 e 50%), é de grande importância o entendimento dos mecanismos envolvidos na sua fisiopatologia, bem como a investigação de novas alternativas terapêuticas. Dessa forma, o objetivo geral desse trabalho foi investigar o envolvimento dos glicocorticoides, eixo hipotálamo-pituitária-adrenal (HPA), microglia e estresse oxidativo na fisiopatologia da depressão, bem como avaliar o efeito tipo-antidepressivo da apocinina. Para isso, primeiramente foi desenvolvido um artigo de revisão abrangendo o envolvimento e relação da microglia e do eixo HPA na depressão. Na sequência, o modelo de administração crônica de corticosterona (ACC) foi realizado para induzir o comportamento do tipo depressivo (CTD) em camundongos, assim como para avaliar seu efeito sobre a expressão proteica do receptor glicocorticoide (GR), fator neurotrófico derivado do encéfalo (BDNF) e seu receptor,TrkB, em estruturas cerebrais (córtex pré-frontal, hipocampo e estriado). Por fim, foi investigado o efeito da apocinina, um antioxidante de origem natural, sobre o CTD induzido pela ACC, bem como seu efeito sobre o perfil oxidativo (enzimas superóxido dismutase, SOD, e catalase, CAT, e sobre os níveis de peróxido de hidrogênio, H_2O_2) após esse protocolo de indução. Através do artigo de revisão foi possível sugerir que os sistemas imunológico e neuroendócrino funcionam de maneira coordenada e a desregulação deles pode estar envolvida em distúrbios psiquiátricos como a depressão, uma vez que a neuroinflamação e o hipercortisolismo são frequentemente observados nessa condição. Após a ACC, foi observado o desenvolvimento de CTD acompanhado de redução do imunoconteúdo de BDNF no córtex pré-frontal e aumento de GR e TrkB no hipocampo dos camundongos. Esse protocolo também foi capaz de reduzir a relação peso adrenal/peso corporal, sugerindo desregulação do eixo HPA bem como a possível relação das vias glicocorticoides/GR e BDNF/TrkB na fisiopatologia da depressão. Ainda, a ACC induziu aumento na atividade da SOD e nos níveis de H_2O_2 e redução na atividade da CAT nas três estruturas cerebrais avaliadas. O tratamento com apocinina foi capaz de reverter tanto o CTD quanto a maioria das alterações oxidativas após o modelo de ACC, sugerindo efeito tipo-antidepressivo. Portanto, o presente trabalho sugere que a depressão apresenta característica “multifisiopatológica”, indica possíveis vias alteradas e ainda sugere a apocinina como um potencial agente o tratamento desse transtorno.

Palavras-chave: Microglia. Eixo HPA. BDNF. Glicocorticoide. Antioxidante.

ABSTRACT

DEPRESSION: PHYSIOPATHOLOGICAL ASPECTS AND THE APOCYNIN ANTIDEPRESSANT-LIKE EFFECT

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CO-ADVISOR: GABRIELA TREVISAN

Depression is considered a public health challenge around the world. According to the World Health Organization, more than 300 million people (approximately 6% of the world population) are affected by this disorder. Considering that depression is closely related to the high rate of death from suicide and that a large number of patients are refractory to any of the available antidepressant agents (between 30 and 50%), understanding the mechanisms involved in its pathophysiology is of great importance, as well as the investigation of new therapeutic alternatives. Thus, the general aim of this work was to investigate the involvement of glucocorticoids, hypothalamic-pituitary-adrenal (HPA) axis, microglia and oxidative stress in the pathophysiology of depression, as well as to evaluate the apocynin antidepressant-like effect. To this end, a review article was first developed covering the involvement and relationship of the microglia and the HPA axis in depression. Subsequently, the chronic corticosterone administration model (CCA) was performed to induce depressive-like behavior (DLB) in mice, as well as to evaluate its effect on the protein expression of the glucocorticoid receptor (GR), brain-derived neurotrophic factor (BDNF) and its receptor, TrkB, in brain structures (prefrontal cortex, hippocampus and striatum). Finally, the effect of apocynin, an antioxidant of natural origin, on the DLB induced by CCA was investigated, as well as its effect on the oxidative profile (enzymes superoxide dismutase, SOD, and catalase, CAT, and on hydrogen peroxide levels, H_2O_2) after this induction protocol. Through the review article it was possible to suggest that the immune and neuroendocrine systems work in a coordinated way and their deregulation may be involved in psychiatric disorders such as depression, since neuroinflammation and hypercortisolism are often observed in this condition. After CCA, the development of DLB was observed, accompanied by a reduction in the BDNF immunocontent in the prefrontal cortex and an increase in GR and TrkB in the hippocampus of mice. This protocol was also able to reduce the adrenal weight / body weight ratio, suggesting dysregulation of the HPA axis as well as the possible relationship of the glucocorticoid / GR and BDNF / TrkB pathways in the pathophysiology of depression. Moreover, ACC induced an increase in SOD activity and H_2O_2 levels and a reduction in CAT activity in the three brain structures evaluated. Apocynin treatment was able to reverse both DLB and most oxidative changes after the CCA model, suggesting an antidepressant-like effect. Therefore, the present study suggests that depression has a “multifisiopathological” characteristic, indicates possible altered pathways and still suggests apocynin as a potential agent for the treatment of this disorder.

Keywords: Microglia. HPA axis. BDNF. Glucocorticoid. Antioxidant.

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DISCUSSÃO GERAL

LISTA DE ABREVIATURAS E SIGLAS

ACC	Administração Crônica de Corticosterona
ACTH	Hormônio Adrenocorticotrópico (do inglês*, adrenocorticotropic hormone*)
ADT	Antidepressivos Tricíclicos
BDNF	Fator Neurotrófico Derivado do Encéfalo (do inglês, *brain-derived neurothophic factor*)
CAT	Catalase
CTD	Comportamento tipo-depressivo
CRH	Hormônio Liberador de Corticotrofina (do inglês, *corticotropin-releasing hormone*)
DSM-V	Manual Diagnóstico e Estatístico de Transtornos Mentais (do inglês, *Diagnostic and Statistical Manual of Mental Disorders*)
MD	Depressão maior
ERNs	Espécies Reativas de Nitrogênio
EROs	Espécies Reativas de Oxigênio
GCs	Glicocorticoides
GPx	Glutationa Peroxidase
GR	Receptor Glicocorticoide
GSH	Glutationa Reduzida
HAM-D	Escala de Avaliação de Depressão de Hamilton (do inglês, *Hamilton Rating Scale for Depression*)
HPA	Hipotálamo-Pituitária-Adrenal
H_2O_2	Peróxido de Hidrogênio
INF-γ	Interferon gama
IL-1	Interleucina 1
IL-1β	Interleucina 1 beta
IL-4	Interleucina 4
IL-6	Interleucina 6
IL-10	Interleucina 10
IMAO	Inibidores da Monoamina Oxidase
IRSN	Inibidores da Recaptação de Serotonina e Noradrenalina
ISRS	Inibidores Seletivos da Recaptação de Serotonina
MDA	Malondialdeído
MR	Receptor Mineralocorticoide
NADPH	Nicotinamida adenina dinucleotídeo fosfato
NGF	Fator de Crescimento Nervoso
NO_2	Dióxido de Nitrogênio
NT-3	Neurotrofina-3
NT-4	Neurotrofina-4
$O_2^{\bullet-}$	Ânion Superóxido
$^1O_2$	Oxigênio Singleto
$OH^{\bullet}$	Radical Hidroxila
ON	Óxido Nítrico
$ONOO^{\bullet}$	Peroxinitrito
p75(NTR)	Receptor de Neurotrofina p75 (do inglês, *p75 neurotrophin receptor*)
PHQ-9	Questionário Sobre a Saúde do Paciente-9 (do inglês, *Patient Health Questionnaire 9*)
SNC	Sistema Nervoso Central
SOD	Superóxido Dismutase

TNF-α	Fator de Necrose Tumoral Alfa
TrkB	Receptor de Tropomiosina Quinase B (do inglês, tropomyosin receptor kinase B)

SUMÁRIO

APRESENTAÇÃO

Os resultados que fazem parte dessa dissertação estão apresentados sob a forma de artigos, os quais encontram-se nos subitens do item **ARTIGO CIENTÍFICO**. As seções Materiais e Métodos, Resultados, Discussão e Referências encontram-se nos próprios artigos e representam a íntegra desse estudo. No item **DISCUSSÃO GERAL** está apresentada uma retomada geral dos resultados obtidos neste trabalho. A seção **REFERÊNCIAS** compreende somente as citações presentes nos itens **INTRODUÇÃO, REFERENCIAL TEÓRICO** e **DISCUSSÃO GERAL** dessa dissertação.

1. INTRODUÇÃO

Transtornos psiquiátricos são caracterizados por uma combinação de pensamentos, percepções, emoções e comportamento atípicos, que afetam a vida do indivíduo bem como a relação do mesmo com outras pessoas (WORLD HEALTH ORGANIZATION, 2019). São exemplos de transtornos psiquiátricos a depressão, a esquizofrenia e outras psicoses, o transtorno afetivo bipolar, a demência, a deficiência intelectual e os transtornos de desenvolvimento, como o autismo (WORLD HEALTH ORGANIZATION, 2019). Estima-se que aproximadamente 450 milhões de pessoas ao redor do mundo sejam acometidas por tais transtornos (ROCHA, HARA e PAPROCKI, 2015). Ainda, o mais prevalente é a depressão maior (DM), a qual atinge mais de 300 milhões de pessoas ao redor do mundo e é considerada um desafio à saúde pública (VOS et al., 2015; WORLD HEALTH ORGANIZATION, 2018).

A DM, também considerada um transtorno afetivo, afeta principalmente o sexo feminino e é caracterizada por humor deprimido, distúrbios alimentares, de sono e de ansiedade (SEEDAT et al., 2009; WORLD HEALTH ORGANIZATION, 2012). Apesar dos avanços no estudo da fisiopatologia da depressão, nenhum dos mecanismos até hoje propostos conseguem explicá-la em sua totalidade (MALHI e MANN, 2018). Entretanto, o estresse psicossocial e/ou emocional já foi identificado como um fator comum à quase todos eles, podendo atingir os mais diversos sistemas do organismo, incluindo o sistema nervoso central (SNC), imunológico e endócrino (ANISMAN e MATHESON, 2005).

O eixo hipotálamo-pituitária-adrenal (HPA) consiste no principal eixo de resposta ao estresse, conectando órgãos periféricos ao SNC (CHROUSOS e GOLD, 1992). Diversos estressores são capazes de ativar esse eixo e o produto final de sua ativação são os glicocorticoides (GCs), conhecidos popularmente como os hormônios do estresse (VILLAS BOAS et al., 2019). A desregulação desse eixo neuroendócrino já foi relacionada à uma variedade de distúrbios psiquiátricos incluindo a depressão, uma vez que o estresse crônico é capaz de induzir a hiperativação do mesmo (ROY et al., 1988).

Situações estressantes também são capazes de ativar a microglia. Componentes do sistema imune, as células microgliais são importantes tanto na proteção quanto no desenvolvimento e funcionamento do cérebro (TREMBLAY, et al., 2013). Frente a algum estímulo, essas células são ativadas e podem liberar diversos

mediadores inflamatórios e oxidativos, os quais também são sugeridos como participantes da DM (HAQUE, et al., 2018). Além disso, o envolvimento microglial também é sugerido baseado em relatos de que, no cérebro de pacientes depressivos, a microglia encontra-se extensamente ativada (RÉUS et al., 2015). Entretanto, a relação desse sistema imune com o sistema neuroendócrino na DM ainda é pouco esclarecida.

A neuroplasticidade e a neurogênese são outros eventos possivelmente alterados pelo estresse e envolvidos na fisiopatologia do transtorno depressivo (HUANG e REICHARDT, 2003). O fator neurotrófico derivado do encéfalo (BDNF) é essencial para estes processos e sua infrarregulação já foi observada em estruturas límbicas, como o córtex pré-frontal e hipocampo, após a exposição a condições estressantes (AUTRY e MONTEGGIA, 2012). Essa desregulação do BDNF pode levar à atrofia de neurônios hipocampais com consequente redução da neurogênese (DUMAN, 2002). Além disso, níveis mais baixos de BDNF foram observados no soro de pacientes depressivos e, em contrapartida, a suprarregulação dessa neurotrofina foi observada após o tratamento crônico com antidepressivos (RUSSO-NEUSTADT, BEARD e COTMAN, 1999; OTTE et al., 2016).

Estudos também tem sugerido a existência de um desequilíbrio característico entre a produção e neutralização de espécies reativas de oxigênio (EROs) e de nitrogênio (ERNs) em pacientes depressivos (WIGNER et al., 2017). Tanto o acúmulo de mediadores inflamatórios quanto de EROs podem prejudicar importantes estruturas cerebrais relacionadas ao humor, incluindo o córtex pré-frontal, hipocampo e estriado, originando déficits cognitivos e sintomas psiquiátricos (DUSI et al., 2015). Ademais, já foi observado aumento nos níveis séricos de marcadores oxidativos como óxido nítrico (ON) e malondialdeído (MDA) em pacientes depressivos, sugerindo a participação de espécies oxidativas na fisiopatologia da DM (MAES et al., 2013).

Como mencionado anteriormente, o estresse corresponde a um fator comum aos mecanismos fisiopatológicos sugeridos para a DM. Neste contexto, a maioria dos modelos de depressão em animais são baseados na exposição a uma variedade de agentes estressores. A administração crônica de corticosterona (ACC), principal hormônio glicocorticoide dos roedores, é um modelo amplamente utilizado para indução do comportamento do tipo depressivo (CTD) na pesquisa básica (DAVID et al., 2009; RAINER et al., 2012). Apesar de aceito e difundido mundialmente, os mecanismos subjacentes ao modelo de ACC ainda necessitam esclarecimentos. Da

mesma forma, é necessária a investigação de novas rotas envolvidas e passíveis de modulação na DM, a fim de que alternativas terapêuticas sejam desenvolvidas para tratar essa epidemia silenciosa.

1.1. REFERENCIAL TEÓRICO

1.1.1. Depressão

A DM consiste em um transtorno do humor comum, o qual está intimamente relacionado à incapacidade e redução da qualidade de vida (SKOOG, 2011). Segundo a Organização Mundial da Saúde, esse distúrbio atinge mais de 300 milhões de pessoas ao redor do mundo (WORLD HEALTH ORGANIZATION, 2018). Isso equivale a cerca de 6% da população mundial, evidenciando uma prevalência de proporções epidêmicas com projeções preocupantes (GREENBERG et al., 2015). Além disso, a depressão corresponde a um dos transtornos mentais com maior risco de suicídio (CHESNEY, GOODWIN e FAZEL, 2014). Estima-se que mais de 50% dos 800.000 casos de suicídios relatados por ano em todo o mundo ocorra durante um episódio depressivo (WORLD HEALTH ORGANIZATION, 2016). No Brasil, aproximadamente 5,8% da população é acometida por essa desordem, o que representa cerca de 11,5 milhões de brasileiros (WORLD HEALTH ORGANIZATION, 2015). Além do mais, o Brasil ocupa a oitava posição no *ranking* mundial quanto à ocorrência de suicídios (WORLD HEALTH ORGANIZATION, 2012). A depressão afeta mais as mulheres, numa proporção de duas a três vezes mais quando comparada ao sexo masculino (SEEDAT et al., 2009). Considerando ambos os sexos, a idade média de início do transtorno depressivo é 25 anos e o pico de risco para o seu desenvolvimento varia entre o final da adolescência e o início dos 40 anos (BROMET et al., 2011).

Tradicionalmente, a depressão é definida como um transtorno afetivo complexo e heterogêneo caracterizado por prejuízo afetivo, cognitivo e fisiológico (ATHIRA et al., 2018). Dentre os sintomas apresentados pelos pacientes, observam-se alterações no humor, como irritabilidade e tristeza, distúrbios do sono e apetite, fadiga, anedonia e sentimento de culpa exagerada (KESSLER et al., 2003; CHIU et al., 2015; BAI et al., 2018). Com característica multifatorial, a depressão pode ser desencadeada tanto por fatores genéticos quanto ambientais. Isso, somado à heterogeneidade de sintomas que podem ser observados, dificulta o diagnóstico do transtorno depressivo, o qual é exclusivamente clínico (VILLAS BOAS et al., 2019).

O Manual Diagnóstico e Estatístico de Transtornos Mentais (do inglês, *Diagnostic and Statistical Manual of Mental Disorders*, DSM) é um instrumento elaborado pela Associação Psiquiátrica Americana (do inglês, *American Psychiatric Association,* APA) que auxilia no diagnóstico, tratamento e pesquisa de distúrbios de

humor como a depressão. Em sua quinta edição, lançada em 2013, são expostos alguns critérios diagnósticos para a DM que incluem: exibir 5 ou mais sintomas como humor deprimido, diminuição do interesse ou prazer na maioria das atividades diárias, perda ou ganho excessivo de peso, assim como alterações no apetite, insônia ou hipersonia, agitação ou retardo psicomotor, fadiga ou perda de energia frequente, sentimento de inutilidade ou culpa excessiva, dificuldade de concentração e de tomada de decisão, pensamento recorrente de morte e ideação suicida, sendo que pelo menos um dos sintomas é o humor depressivo ou anedonia; apresentar prejuízo social, profissional ou em outras áreas importantes na vida do indivíduo; e não atribuição desses sintomas a efeitos fisiológicos de substâncias ou a outra condição médica. Ainda, o DSM-V classifica a DM de acordo com a frequência (episódio único ou recorrente), gravidade (leve, moderada ou grave), presença de características psicóticas e estado de remissão (em remissão parcial ou em remissão completa). Além disso, esse manual destaca a importância do diagnóstico diferencial da DM e outros transtornos assim como de períodos de tristeza (AMERICAN PSYCHIATRIC ASSOCIATION, 2014).

Para facilitar a identificação dos critérios anteriormente citados, existem questionários que podem ser aplicados aos pacientes (AROS e YOSHIDA, 2009). Dentre eles destacam-se a Escala de Avaliação de Depressão de Hamilton (do inglês, *Hamilton Rating Scale for Depression*, HAM-D), o Inventário de Depressão de Beck-II (do inglês, *Beck-II Depression Inventory*) e o Questionário Sobre a Saúde do Paciente-9 (do inglês, *Patient Health Questionnaire 9*, PHQ-9) (GUERRA et al., 2018). Todos eles visam avaliar a história clínica do paciente, auxiliando no diagnóstico correto e possibilitando o tratamento adequado.

Apesar das possibilidades de diagnóstico acima citadas, esse transtorno permanece muitas vezes não diagnosticado. Isso está relacionado com o estigma social que ainda assombra pacientes depressivos, assim como aos recursos limitados à saúde mental. Um levantamento feito em 2014 revelou que, quase metade da população mundial vive em países com apenas dois psiquiatras para cada 100.000 pessoas (SMITH, 2014). Ademais, esta desordem apresenta curso altamente variável com possibilidade de diferentes respostas aos tratamentos. Isso relaciona-se com a alta taxa de refratariedade apresentada pelos pacientes depressivos, onde de 30 a 50% deles não respondem a nenhum antidepressivo disponível na prática clínica (BSCHOR et al., 2012).

1.1.2.Mecanismos fisiopatológicos da depressão

Diferentes mecanismos já foram propostos na tentativa de explicar a fisiopatologia da depressão. A hipótese pioneira é a das monoaminas, a qual sugere um déficit na neurotransmissão monoaminérgica (neurotransmissores dopamina, serotonina e noradrenalina) de pacientes depressivos. Postulada por Schildkraut em 1965, a hipótese monoaminérgica serviu como base para o desenvolvimento das principais classes de antidepressivos, como os tricíclicos (ADT, ex: amitriptilina, imipramina, nortriptilina), inibidores da monoamina oxidase (IMAO, ex: fenelzina, a isocarboxazida, tranilcipromina), assim como inibidores da recaptação de serotonina e noradrenalina (IRSN, duloxetina, venlafaxina, desvenlafaxina) e inibidores seletivos da recaptação de serotonina (ISRS, fluoxetina, citalopram, paroxetina, sertralina) (SCHILDKRAUT, 1965; STAHL, 2013).

No entanto, a teoria das monoaminas exibe muitas limitações como o não esclarecimento da origem dos distúrbios monoaminérgicos, assim como a alta taxa de refratariedade dos pacientes aos antidepressivos baseados nessa hipótese (VISMARI; ALVES; PALERMO-NETO, 2008). Nesse contexto, estudos têm sugerido outros sistemas como participantes na fisiopatologia da DM, como o eixo HPA, sistema imune, sistema neurotrófico e estresse oxidativo, os quais serão brevemente discutidos abaixo.

Até os dias atuais, o achado mais robusto sobre a DM consiste em sua estrita relação com anormalidades na resposta frente ao estresse (JURUENA et al., 2018). De fato, transtornos de humor são comumente desencadeados ou exacerbados por eventos estressantes agudos ou crônicos (GOLD e CHROUSOS, 2002). Estresse é definido como qualquer evento ou experiência que ameace a capacidade de um indivíduo de lidar e se adaptar (VILLAS BOAS et al., 2019). Comum a quase todos os mecanismos fisiopatológicos sugeridos para a depressão, o estresse é capaz de atingir os mais diversos sistemas, como o SNC, sistema imunológico e sistema endócrino (ANISMAN e MATHESON, 2005). O eixo HPA consiste no principal sistema de resposta ao estresse, conectando órgãos periféricos ao SNC (CHROUSOS e GOLD, 1992). Diferentes estressores são capazes de ativar este eixo, estimulando o núcleo paraventricular do hipotálamo a secretar o hormônio liberador de corticotrofina (CRH), liberando-o na glândula pituitária (ou hipófise). Lá, o CRH estimula a liberação de hormônio adrencorticotrópico (ACTH), que por sua vez ativa a secreção

adrenocortical de glicocorticoides (GCs), o cortisol, em humanos, e a corticosterona, em roedores (KATO et al, 2013; VILLAS BOAS et al, 2019).

A desregulação deste eixo já foi relacionada à uma ampla variedade de distúrbios psiquiátricos incluindo a depressão. Nessa condição, o estresse crônico é frequentemente presente e capaz de induzir a hiperativação do eixo HPA (ROY et al., 1988). Com isso, há um aumento na liberação de glicocorticoides acompanhada de prejuízo na retroalimentação negativa (*feedback* negativo) exercida por estes hormônios, incitando a constante secreção e perpetuando os níveis elevados dos mesmos (GAFFEY et al., 2016). Hinkelmann e colaboradores (2009) demonstraram essa atividade exacerbada do eixo HPA em pacientes com depressão. Nestes indivíduos, foram observadas anormalidades como aumento das glândulas pituitária e adrenal, além de hipercortisolismo e alterações cognitivas (HINKELMANN et al., 2009). Ainda, pacientes depressivos já demonstraram níveis aumentados de CRH no líquido cefalorraquidiano bem como secreção aumentada de ACTH, hormônio que já foi testado em ratos e induziu CTD via receptores NMDA (NEMEROFF et al., 1984; CARROLL et al., 2007; TOKITA et al., 2012). Mais dados sobre o funcionamento do eixo HPA bem como seu envolvimento na depressão estão descritos e discutidos no Manuscrito 1 (item 3.1).

Disfunções imunes também vêm sendo fortemente relacionadas a transtornos psiquiátricos e neurológicos (FRICK; WILLIAMS e PITTENGER, 2013). As células microgliais correspondem aos macrófagos residentes no SNC que, além de defesa, desempenham papel importante no desenvolvimento e funcionamento do cérebro (TREMBLAY, et al., 2013). A microglia exibe diferentes morfologias. No repouso, mostra-se altamente ramificada. Entretanto, quando a homeostase cerebral é perturbada, seja por algum dano ou infecção, tais células assumem a forma ativada, apresentando formato ameboide, com maior mobilidade e capacidade fagocítica (DAVALOS, et al., 2005; NIMMERJAHN; KIRCHHOFF e HELMCHEN, 2005; HELLWIG; HEINRICH e BIEBER, 2013). Além disso, essas células podem assumir dois perfis de ativação: M1 e M2. A primeira produz mediadores pró-inflamatórios, como interleucina 1-beta (IL-1β), interleucina 6 (IL-6) e fator de necrose tumoral alfa (TNF- α), bem como EROs e ERNs. Já a segunda é responsável por produzir fatores neuroprotetores como as interleucinas anti-inflamatórias 4 (IL-4) e 10 (IL-10) e neurotrofinas, como o BDNF (HAQUE, et al., 2018).

Considerando o papel ambíguo da microglia, desempenhando tanto neurotoxicidade quanto neuroproteção, é necessária a atividade coordenada entre os dois possíveis fenótipos de ativação microglial. Além disso, uma vez que a microglia é a principal fonte de mediadores inflamatórios no cérebro, seu envolvimento também é sugerido na fisiopatologia da depressão (MAJIDI; KOSARI-NASAB e SALARI, 2016). Já foi relatado que, no cérebro de pacientes com DM e com doença de Alzheimer, a microglia encontra-se extensamente ativada (RÉUS et al., 2015; SALTER e STEVENS, 2017). Consequentemente, é comum que essas doenças sejam acompanhadas de produção significativa de EROs e citocinas inflamatórias.

Tanto o acúmulo de mediadores inflamatórios quanto de EROs podem prejudicar importantes estruturas cerebrais relacionadas ao humor, como o córtex pré-frontal, hipocampo e estriado, originando déficits cognitivos e sintomas psiquiátricos (DUSI et al., 2015). Além do mais, pacientes depressivos frequentemente exibem níveis séricos aumentados de citocinas pró-inflamatórias como IL-1, IL-6, interferon gama (INF-γ) e TNF-α e modelos animais experimentais já sugeriram que a inibição da ativação microglial melhora o CTD em camundongos (DOWLATTI et al., 2010; ZHAO et al., 2016). Assim como para o eixo HPA, uma revisão mais completa sobre a microglia e seu envolvimento nos mecanismos subjacentes à depressão estão presentes no Manuscrito 1 (item 3.1).

Outros eventos alterados pelo estresse e possivelmente envolvidos na fisiopatologia do transtorno depressivo são a neurogênese e a neuroplasticidade (HUANG e REICHARDT, 2003). Entende-se por neurogênese a geração de novos neurônios que ocorre continuamente mesmo no encéfalo adulto, ao passo que a neuroplasticidade é entendida como o conjunto de modificações e reorganizações que ocorrem após algum estímulo (BEAR; CONNORS e PARADISO, 2017; VILLAS BOAS, 2019). Para a ocorrência desses processos, é essencial a participação de fatores neurotrófico, dos quais destaca-se o BDNF (BJÖRKHOLM e MONTEGGIA, 2016).

O BDNF pertence à uma família de neurotrofinas que inclui o fator de crescimento nervoso (NGF), neurotrofina-3 (NT3) e a neurotrofina-4 (NT4), e consiste na neurotrofina mais abundante do cérebro (AL-QUDAH ET AL., 2015). Esse fator neurotrófico é sintetizado na forma de seu precursor, proBDNF, o qual pode ser armazenado nos dendritos ou nos axônios até que seja clivado à proteína BDNF madura (LESSMANN; GOTTMANN e MALCANGIO, 2003). Quanto à sua liberação,

ela ocorre de maneira dependente da atividade, como uma mistura de proBDNF e BDNF (PANG et al., 2004). A síntese de proBDNF ocorre tanto em condições fisiológicas quanto patológicas e essa forma imatura apresenta maior afinidade de ligação ao receptor de neurotrofina p75 (do inglês, *p75 neurotrophin receptor*, p75(NTR)), induzindo apoptose (FRIEDMAN, 2010). Diferentemente do proBDNF, o BDNF liga-se preferencialmente nos receptores de tropomiosina quinase B (do inglês, *tropomyosin receptor kinase B*, TrkB) e promove sobrevivência celular (VOLOSIN et al., 2006). Outras funções críticas do SNC também são associadas à essa neurotrofina tais como maturação neuronal, formação de sinapses e plasticidade sináptica (PARK e POO, 2013). Além disso, o BDNF tem sido relacionado a diferentes doenças psiquiátricas e transtornos do humor como a depressão (CASTRÉN e KOJIMA, 2017).

Como mencionado anteriormente, o BDNF é essencial para a homeostase neuronal. Após a exposição a condições estressantes, sejam elas agudas ou crônicas, sua expressão é frequentemente infrarregulada em estruturas límbicas, como córtex pré-frontal e hipocampo (SMITH et al., 1995; PIZARRO et al., 2004; AUTRY e MONTEGGIA, 2012). Esta desregulação do BDNF pode levar à atrofia de neurônios hipocampais com consequente redução da neurogênese, sendo este um achado comum na depressão (DUMAN, 2002). Ainda, os níveis séricos desta neurotrofina vêm sendo considerados um possível biomarcador de DM. Isto é sugerido uma vez que estudos clínicos demonstraram redução significativa de BDNF no soro de pacientes com esse transtorno (BRUNONI; LOPES e FREGNI, 2008; SEN; DUMAN e SANACORA, 2008). Redução na expressão de RNAm e nos níveis proteicos de BDNF também foram encontrados em análises *post mortem* do cérebro de pacientes depressivos que cometeram suicídio (DWIVEDI et al., 2003).

Por outro lado, uma suprarregulação de BDNF foi observada após o tratamento crônico com antidepressivos (RUSSO-NEUSTADT, BEARD e COTMAN, 1999; OTTE et al., 2016). Na pesquisa básica, os resultados corroboram. Schmidt e Duman (2010) demonstraram que a administração periférica de BDNF promove melhora tanto do comportamento quanto da neurogênese hipocampal de camundongos submetidos ao protocolo de estresse crônico imprevisível (SCHMIDT e DUMAN, 2010).

O estresse oxidativo também é sugerido como participante do transtorno depressivo (MICHEL, PÜLSCHEN e THOME, 2012). Esse evento é caracterizado pelo desequilíbrio entre a produção espécies oxidantes, principalmente EROs, e a

capacidade do organismo de neutralizá-las, podendo originar um dano oxidativo progressivo (VALKO et al., 2007). EROs, como por exemplo o ânion superóxido ($O_2^{\bullet}$), peróxido de hidrogênio (H_2O_2), radical hidroxila ($OH^{\bullet}$), oxigênio singleto ($^1O_2$), e ERNs como o óxido nítrico (ON), peroxinitrito ($ONOO^{\bullet}$) e dióxido de nitrogênio (NO_2), são produtos do metabolismo celular normal que, em baixas concentrações, participam de diversas respostas celulares como sinalização celular e resposta a agentes infecciosos (VALKO et al., 2006). Entretanto, quando em níveis elevados, essas espécies podem reagir com ácidos graxos, proteínas e com o DNA, comprometendo suas funções (MAES et al., 2011).

As espécies reativas podem ser produzidas tanto por fontes exógenas quanto endógenas (HALLIWELL, 2011). Dentre as fontes externas estão a radiação UV, o tabagismo, o consumo de drogas, dentre outras (MARTELLI e NUNES, 2014). Já como principais fontes endógenas destacam-se as enzimas da cadeia respiratória mitocondrial, a via metabólica do citocromo P450, peroxissomos e ativação de células inflamatórias (VALKO et al., 2004; VALKO et al., 2006). Sistemas enzimáticos como xantina oxidase, mieloperoxidase, lipoxigenase e nicotinamida adenina dinucleotídeo fosfato (NADPH) oxidase também são potenciais geradores de EROs. Além disso, o complexo enzimático NADPH oxidase consiste em uma das principais fontes de $O_2^{\bullet-}$ e é amplamente expresso em células fagocíticas do sistema imunológico, assim como em células endoteliais vasculares e células do tecido renal (GEISZT e LETO, 2004).

Uma vez que as espécies reativas são constantemente formadas, sistemas antioxidantes protegem o organismo de possíveis danos oxidativos. O mecanismo pelo qual essa proteção é executada pode ter característica enzimática ou não enzimática (VALKO et al., 2004). As principais defesas enzimáticas incluem a glutationa peroxidase (GPx), a superóxido dismutase (SOD) e a catalase (CAT). Dentre essas enzimas, uma das mais efetivas como antioxidante é a SOD, uma vez que catalisa a dismutação do $O_2^{\bullet-}$ em oxigênio molecular e H_2O_2. Contudo, apesar de não ser um radical livre, o H_2O_2 é extremamente reativo, devendo ser convertido a produtos menos tóxicos por enzimas como a CAT e/ou peroxidases (BARBOSA et al., 2010). Por outro lado, as defesas não enzimáticas compreendem antioxidantes tiólicos como a glutationa reduzida (GSH), bem como as vitaminas C e E e o composto carotenoide β-caroteno (VALKO et al., 2007).

O envolvimento do estresse oxidativo em doenças neurodegenerativas e psiquiátricas vêm ganhando enfoque, uma vez que o cérebro é considerado

extremamente sensível aos danos oxidativos (HALLIWELL, 2006). Estudos em ratos e camundongos têm demonstrado que, após protocolo de indução à depressão, o estresse oxidativo está presente (SULAKHIYA et al., 2016; IBI et al., 2017; THAKARE et al., 2017). Essa afirmação baseia-se na observação de aumento nos níveis de marcadores oxidativos, como por exemplo o malondialdeído (MDA), assim como uma alteração nos sistemas de defesa antioxidante, como as enzimas SOD e CAT no córtex cerebral, hipocampo e estriado (AHMAD et al., 2010; YANG et al., 2018). Além disso, a administração de compostos naturais com características antioxidantes foi capaz de produzir melhora comportamental em camundongos através de aumento nas defesas antioxidantes endógenas (DI LORENZO et al., 2016; NABAVI, et al. 2018). Ainda, a co-administração de vitamina C potencializou doses sub-efetivas de fluoxetina em camundongos (BINFARÉ et al., 2009).

Evidências clínicas também sugerem a existência de um desequilíbrio característico entre a produção e neutralização de EROs em pacientes depressivos (WIGNER et al., 2017). Já foi reportado que tais indivíduos apresentam níveis reduzidos de enzimas e compostos antioxidantes, como vitamina C e E, zinco e coenzima Q10, no plasma e/ou soro, além de aumento sérico de ON e MDA (MAES et al., 2013; SALIM, 2014; DU et al., 2016). Além disso, aumento de H_2O_2 também tem sido relatado na DM, assim como a redução nos níveis de GSH em análises *post mortem* do córtex pré-frontal de pacientes com DM (MAES et al., 2011; GAWRYLUK et al., 2011). Ademais, o uso por seis meses de vitamina C como adjuvante ao tratamento com fluoxetina em pacientes pediátricos com DM promoveu diminuição dos sintomas depressivos em comparação ao grupo tratado com fluoxetina e placebo (AMR et al., 2013). Da mesma forma, a co-administração de coenzima Q10 por 8 semanas promoveu melhora dos sintomas clínicos acompanhada de redução dos parâmetros oxidativos e inflamatórios em pacientes com transtorno bipolar na fase depressiva (JAHANGARD et al., 2019).

A apocinina (4-Hidroxi-3-metoxiacetofenona), também chamada de acetovanilona, é outro composto com característica antioxidante que atua como um inibidor do complexo enzimático NADPH oxidase (PETERS, HILTERMANN e STOLK, 2001). Originalmente isolada das raízes da planta medicinal *Picrorhiza kurroa*, a apocinina é pouco tóxica, muito estável e ativa por via oral. Tais características tornam-na uma candidata promissora para estudos que busquem novas alternativas para o tratamento de condições inflamatórias e oxidativas, como a depressão

(JOHNSON et al., 2002; STEFANSKA e PAWLICZAK, 2008). Nesse trabalho, tanto o efeito do tipo-antidepressivo da apocinina, quanto o envolvimento do estresse oxidativo, do eixo HPA, da microglia e da sinalização do BDNF na depressão, foram avaliados.

2. OBJETIVOS

2.1. OBJETIVO GERAL

Investigar o envolvimento dos glicocorticoides, eixo HPA, microglia e estresse oxidativo na fisiopatologia da depressão, bem como avaliar o efeito tipo-antidepressivo da apocinina.

2.2. OBJETIVOS ESPECÍFICOS

- Elaborar um artigo de revisão abrangendo o envolvimento da microglia e do eixo HPA na depressão;
- Caracterizar o CTD (através do teste de suspensão da cauda, dos níveis séricos de Cort e da relação peso adrenal/peso corporal) após ACC em camundongos;
- Avaliar o efeito da ACC sobre a expressão proteica de GR, BDNF e TrkB em estruturas cerebrais (córtex pré-frontal, hipocampo e estriado) de camundongos;
- Investigar o efeito da apocinina sobre o CTD induzido pela ACC através dos testes comportamentais;
- Avaliar o perfil oxidativo após a ACC, bem como após tratamento com apocinina.

3. ARTIGO CIENTÍFICO

3.1. MANUSCRITO 1: Microglia and HPA axis: an overview of participation and relationship on depression pathophysiology

O manuscrito apresentado a seguir consiste em um artigo de revisão, apresentando a seção “Introdução”, “Materiais e Métodos”, seguida do seu desenvolvimento, "Conclusão” e “Referências”. O formato no qual está apresentado foi submetido ao periódico *The World Journal of Biological Psychiatry*.

Microglia and HPA axis: an overview of participation and relationship on depression pathophysiology

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Microglia and HPA axis: an overview of participation and relationship on depression pathophysiology

Objectives: This paper aims to discuss the involvement and relationship between microglia and the hypothalamic-pituitary-adrenal (HPA) axis in the pathophysiology of depression. **Methods:** A search for English language articles was performed using PubMed. **Results:** Depression is considered a public health challenge worldwide and its pathophysiology is not yet fully understood. However, emotional stress is considered a common factor to the mechanisms proposed to explain it. Stressful conditions can activate microglial cells, important immune components of the central nervous system involved in maintaining brain homeostasis. Another system involved in response to stressors is the hypothalamic-pituitary-adrenal (HPA) axis. This axis represents the main stress response system and is responsible for the production of the glucocorticoid hormone (GC). Depressive individuals frequently show changes in both microglial and HPA axis activity. Also, mediators released after microglial activation can activate HPA axis, stimulating the production of GC. Likewise, high levels of the GC are also capable of activating the microglia, generating a vicious cycle. This creates a neurotoxic environment that may be related to the development or worsening of depressive symptoms. **Conclusion:** The reported immune and neuroendocrine systems work in a coordinated manner and their dysregulation may be involved in the pathophysiology of depression.

Keywords: Neuroinflammation; stress; neurobiology; glucocorticoid.

Introduction

Depression is traditionally defined as a complex and heterogeneous mood disorder characterized by affective, cognitive and physiological impairment (Athira et al. 2018). According to the World Health Organization (WHO), 350 million people - 5% of the global population - may present depressive symptoms, from which about 1 million commit suicide annually (WHO, 2012; WHO, 2018). Roughly 25% of people diagnosed with depression are under 19 years old, and nearly 40% of depressive patients do not satisfactorily respond to ongoing therapeutics protocols (Dwivedi 2014). It is related to the lack of elucidation of its pathophysiology since, despite the many mechanisms proposed, it remains poorly understood (Ménard et al. 2015).

The term “glial cells” refers to all non-neuronal cells in the central nervous system (CNS). These cells represent over 90% of the human brain and consist of two main populations: the macroglia, represented by astrocytes and oligodendrocytes, and the microglia, the CNS tissue-resident macrophages (Greter and Merad 2013). Microglial cells detect the first signs of pathogenic invasion or tissue damage. When challenged, they can uphold tissue repair and remodelling. Paradoxically, these immune cells may also exert harmful and pathological processes to the CNS, including in psychiatric diseases (Béchade et al. 2013; Norden et al. 2015). Studies suggest microglial participation in the pathophysiology of depression, mainly due to the release of proinflammatory mediators by these cells when activated (Yirmiya et al. 2015). Also, emotional stress, in addition to being related to depressive disorder, can activate these cells and promote neuroinflammation (Kreisel et al. 2014). Moreover, patients suffering from chronic inflammation often develop depressive symptoms, while depressed individuals often exhibit increased levels of circulating cytokines (Brites and Fernandes 2015). Moreover, a study developed using post-mortem brain samples from suicidal

patients with a history of depression showed morphological changes in the microglia (Steiner et al. 2008).

In addition to activating the microglia and the development of depression, acute, chronic or repeat emotional stress is also capable of activating the hypothalamic-pituitary-adrenal (HPA) axis (Hansson et al. 2015). This axis is one of the main endocrine systems that maintain the body's homeostasis in the face of stressful challenges (Juruena 2014). After successive stages, the ending products of its stimulation are glucocorticoid hormones (GCs), which also have a suggested role in the pathophysiology of depression (Keller et al. 2016). Depressive patients often demonstrate HPA axis hyperactivity, enlarged pituitary and adrenal glands and hypercortisolism (Pariante and Lightman 2008; Hinkelmann et al. 2009). Moreover, patients with chronic depression or treatment-resistant depression usually have twice serum cortisol values compared with healthy individuals (Juruena et al. 2006). Additionally, a link between HPA axis and immune system has also succinctly suggested (Elenkov et al. 1999).

This simultaneous involvement of the CNS and the immunological system in stress responses, as well as their participation in depression, indicates the broad spectrum in which this disease may manifest (Ménard et al. 2015). Thus, this review aims to understand and clarify the relationship and the role of microglia and HPA axis in depression.

Materials and Methods

This review article was developed based on a search for English language articles in PubMed.

Microglia

Microglia are the brain-resident macrophages which account for 15% of total CNS cells

and act as the first active immune barrier in the CNS (Müller et al. 2015; Stratoulias et al. 2019). These cells are broadly present throughout the brain and the spinal cord and are suggest not only to be involved in the CNS immune defence but are being also responsible for maintaining neuronal homeostasis (Ginhoux et al. 2013). Their number and location may vary according to different species: in mice there are more microglia in the grey matter than in the white one, while in humans the opposite is found. Although microglia are found in all the brain, their roles differ in specific regions and develop unique characteristics based on tissue-specific molecular signals. Thus, not represent a uniform entity, but rather a heterogeneous community with subpopulations being distinguishable by the function they perform (Hanisch 2013; Gertig and Hanisch 2014; Neiva et al. 2014; Doorn et al. 2015; Wohleb 2016).

Under physiological conditions, when there is no tissue injury or invasion by pathogens, microglia are in its inactive conformation, with highly ramified branching processes emerging from a cell body of small dimensions (Fu et al. 2014). However, these branches of “inactive” microglia seem to constantly sweep the microenvironment as a constant inspection, supporting the idea that these cells are highly dynamics and act as the first line of defence of the CNS (Nimmerjahn et al. 2005; Biber et al. 2007; Norden et al. 2015; Alimamy et al. 2018). In case of brain infection or damage, microglia may transform into its active phagocytic form, migrating by chemotaxis and concentrating at the site of injury (Stence et al. 2001). The retracted branching process and amoeboid morphology are the main characteristics of the activated microglia (Gehrmann et al. 1995). In addition, this state of activation can be identified by the release of pro or anti-inflammatory molecules and by their increased ability to phagocyte apoptotic cells and debris, representing a different phenotype from inactive form (Gehrmann et al. 1995; Biber et al. 2007).

Microglia can produce a variety of chemical mediators, both neurotoxic and neuroprotective. Two kinds of microglia activation modes have been described: classical M1 and alternative M2 (Alimamy et al. 2018). Usually, after CNS damage, an inflammatory response coordinated by the microglia M1 phenotype begins. This activation generates oxidative and inflammatory mediators, including reactive oxygen species (ROS), arachidonic acid derivatives, prostaglandin E2 (PGE2), proteases as well as release chemokines and pro-inflammatory cytokines (Caldeira et al. 2014; Bhatia et al. 2016; Mayer et al. 2016). It can be included the interleukin 1 beta (IL-1β), tumour necrosis factor (TNF-α), IL-6 and interferon-γ (IFN-γ) (González et al. 2014).

In short time, the M1 phenotype action is beneficial and subsequently is resolved by the activity of the M2 phenotype, which attenuates the inflammation generated by the classical microglia activation (Shechter et al. 2013; González et al. 2014). M2-activated microglia produce anti-inflammatory effects releasing cytokines such as interleukin 4 (IL-4), interleukin 10 (IL-10), transforming growth factor β (TGF-β), and IL-13, which are important to restore the tissue homeostasis (Littlefield et al. 2015; Mayer et al. 2016). Moreover, these microglial cell subtype also produce several neuroprotective factors, such as neurotrophins, including brain derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF) and nerve growth factor (NGF) (Suzumura 2013). Therefore, the functioning of M1/M2 phenotypes must happen in a coordinated way since dysregulation may be related to impairment of neuronal function and thereafter development of CNS diseases related to neuroinflammation and oxidative stress (Liu et al. 2017; Salim 2017).

Microglia and depression

As described above, according to the microglial activation subtype, these cells may

play a dual role in the brain, acting as neuroprotective or neurotoxic. In this context, microglia involvement has been described in a myriad of pathologies including depression, which is defined by some authors as a type of microglial disease (Borsini et al. 2015; Han et al. 2019). This is mainly suggested due to the exaggerated inflammatory response after M1 classical activation of these cells by both peripheral and central triggers (L. Zhang et al. 2018). Peripherals include mediators produced by inflamed or damaged cells, such as cytokines that, under basal conditions, has limited interaction with the CNS (Schwartz and Shechter 2010). However, under neuroinflammatory or stressful condition these peripheral molecules can enter the brain and activate the microglia (Thurgur and Pinteaux 2019). On the other hand, the central sources of possible microglial activators include inflammatory mediators (cytokines, chemokines and prostaglandins) and oxidants agents (ROS and nitric oxide), neurotransmitters, neuromodulators as well as stress-associated hormones and other mediators released after neuronal damage (Yirmiya et al. 2015; Thurgur and Pinteaux 2019). Di Filippo et al. (2013) demonstrated that central and peripheral inflammation activates hippocampal microglia in mice and that this possibly negatively influences cognition and behaviour (Di Filippo et al. 2013). Thus, regardless of their source, once in the brain, these triggers can promote M1 microglial activation, stimulating the secretion of other proinflammatory and oxidative mediators. These sequential events provide a neurotoxic environment which perhaps is related to both microglial activation and the development of CNS diseases, as depression (Réus et al. 2015).

In humans and experimental animal models, stress is described as a determinant factor in the development of depression (Kendler et al. 2003; Goshen and Yirmiya 2009; Kreisel et al. 2014). Studies suggests that acute and chronic stress

induces microglial activation and inflammation in the brain as well as peripherally (Rohan et al. 2013; Rohleder 2014; Calcia et al. 2016). Additionally, exposure to inflammatory cytokines such as TNF-α, IFN-γ and IL-1β or cytokine inducers, for example lipopolysaccharides (LPS), also leads to behavioural changes in humans and rodents (Liu et al. 2017). These and other inflammatory mediators such as acute phase proteins (C-Reactive Protein - CRP - and others), adhesion molecules, and prostaglandins (PGs) have also been found in high concentrations in peripheral blood, CNS, and cerebrospinal fluid in depressed patients (Miller et al. 2009; Norman et al. 2009; Dowlati et al. 2010). Other reports have noticed that depressive individuals presented heightened serum levels of IL-1β, IL-6, IL-8, IL-12 and TNF-α, synchronously to reduced IL-10 levels (Schiepers et al. 2005; O'Brien et al. 2007; Song et al. 2009).

As the main source of proinflammatory cytokines in the brain, microglia is important in regulating hippocampal neurogenesis, event that is negatively influenced by proinflammatory cytokines and is impaired in depressive condition (Dowlati et al. 2010). Chronic stress also is suggested as a detrimental factor to hippocampal neurogenesis and antidepressant treatment tends to normalize this phenomenon (Mahar et al. 2014). In this context, some authors demonstrated that chronic stress, initially, induce microglial proliferation and activation but, sequentially, induce apoptosis, dystrophy and a reduction in the number of these cells (Kreisel et al. 2014; Gao et al. 2019). These late effects, especially in the hippocampus, suggest that microglial loss is also related to depression, disorder in which hippocampal volume may also be reduced (Bremner et al. 2000; Kreisel et al. 2014). This emphasizes the dynamic and bi-directional character of microglia as well as the need to intensify studies on its role in depression.

Gut microbiota effects on microglia and the development of depression also have been suggested but remained uncertain until now (Singhal and Baune 2017). Intestinal microbiota is responsible for mediating the bidirectional interaction between central and enteric nervous system through the gut-brain-axis (Xie et al. 2018). This communication connects the cognitive and emotional centres in the brain with intestinal functions and intestinal microflora dysbiosis contributes to the development of neurological disorders in animals and humans (Carabotti et al. 2015). Evidences suggest that patients suffering from depression have different gut microbiota composition than healthy individuals, and the faecal microbiota transplantation of depressed patients leads to a depressive-like behaviour in germ-free mice (Naseribafrouei et al. 2014; Zheng et al. 2016). Erny et al. (2015) also provided evidence for that connection by ways of modulation of microglial associated immune networking. It was observed that germ-free mice showed deficits in microglial functions as well as reduction in their numbers, leading to impaired immunological responses, which affects neural circuitry, a potential factor for the onset of depression (Erny et al. 2015). Gut microbiota is, thereby, suggested to play a role in depression, especially during early life, through modifications of microglial activity in brain. However, advanced researches are required to better establish this contemporary hypothesis.

Based on the findings cited above it may be suggested that modulation of microglial activity is an alternative for antidepressant treatment. Many studies have shown that inhibiting microglial activation produces improvement in depressive-like behaviour. Zhao et al. (2016) demonstrated that pioglitazone, a hypoglycaemic drug, can reduce the depressive-like behaviour of mice exposed to chronic stress by reducing expression of M1 microglial markers and increase expression of M2 in the hippocampus (Zhao et al. 2016). Zhang et al. (2019) also demonstrated that chronic

treatment with minocycline, a microglial inhibitor, improved the depressive like-behaviour in rats exposed to chronic unpredictable mild stress. This effect was associated with suppression of M1 response and restoration of expression of BDNF, its receptor TrkB, and of glial fibrillary acidic protein, GFAP, astrocyte marker (Zhang et al. 2019). In addition, Su et al. (2015) investigated the effect of fluoxetine and S-citalopram, selective serotonin reuptake inhibitors (SSRIs) used as first line in depression treatment, in BV-2 cells, a microglial cell line. They showed that these drugs significantly reduced the classical M1 activation while increasing the alternative activation M2 of these cells. Thus, the authors suggest that this immune system modulation may be partially involved in the therapeutics effects of these widely used antidepressants (Su et al. 2015).

To assess the microglial status, despite IL-6, IL-1β, TNF-α and nitric oxide synthase inducible (iNOS) are considered markers of M1 microglial activation, whereas IL-4, IL-10 and TGF-β markers of M2 microglial activation, it is interesting to evaluate more than the levels of these molecules (Duan et al. 2019). In this context, other techniques are used to identify microglial activity in depression and other neuropathologies. One of them is the positron emission tomography (PET), the one method for detecting microglial activation in brain of living individuals (Suzuki et al. 2019). For this, translocator protein 18kDa (TSPO) ligands is employed once this protein is extremely expressed in the brain mainly in activated microglia and reactive astrocytes, comprising a good biomarker of neuroinflammation (Chen and Guilarte 2008). In the healthy brain, TSPO expression levels appear to be reduced, but are markedly upregulated during neuropathological events (Rupprecht et al. 2010). This justify the assessing the binding of TSPO ligands marked by PET imaging techniques in order to visualize and quantify various neuroinflammatory conditions. Setiawan et

al. (2015) demonstrated that during depression there is an elevation on TSPO density on different brain regions and this correlates positively with the severity of the symptoms (Setiawan et al. 2015). On the other hand, no correlation was found between TSPO binding and peripheral markers of inflammation, reinforcing the necessity to investigate beyond cytokines level.

Another alternative to assess the microglial involvement is applying immunohistochemistry to evaluate markers of microglia activated like as ionized calcium-binding adapter molecule 1 (Iba-1) and CD11b (Ahmed et al. 2007). Wang et al. (2018) founded highest expression of these markers in rats submitted to stress, which also demonstrated depressive-like behaviour on the forced swimming test (FST) (Wang et al. 2018). Similar results were found by Alzarea and Rahman (2018), where LPS-injection, in addition to increasing immobility time in FST and tail suspension test (TST), increased Iba-1 expression in mice prefrontal cortex and hippocampus, structures related to mood disorders (Alzarea and Rahman 2018). Moreover, mRNA and protein expression of CD11b in rats also were higher after stress protocol, where the animals also developed depressive like-behaviour in FST and TST. These alterations returned to levels like the control group after treatment with minocycline, a second-generation tetracycline capable of crossing the blood-brain-barrier and inhibiting microglial activation (Zhang et al. 2019). A summary of the relationship between microglia and depression is illustrated in Figure 1.

HPA axis

The hypothalamic-pituitary-adrenal (HPA) axis modulates various physiological activities such as fertility and immunity, and is the main stress response system, connecting peripheral organs to the CNS (Chrousos and Gold 1992; Demorrow 2018).

Its activity is initiated by the hypothalamic secretion of corticotropin-releasing hormone (CRH), also known as corticotropin-releasing factor (CRF), which is released into the pituitary gland. There, adrenocorticotropic hormone (ACTH) secretion is stimulated, which in turn activates adrenocortical synthesis and secretion of GCs, cortisol in humans, and corticosterone in rodents (Juruena et al. 2004; Kato et al. 2013; Spencer and Deak 2016; Villas Boas et al. 2019). After released by adrenal glands, these GCs can act on mineralocorticoid receptor (MR) and glucocorticoid receptor (GR), both belonging to the nuclear receptor class (Reul and Kloet 1985). These receptors are expressed in different tissues and are responsible for mediating different physiological functions (Gjerstad et al. 2018). Under basal conditions, the circadian rhythm coordinates the release of GCs by the adrenal glands, showing peak levels in the active phase, morning in humans and beginning of night in nocturnal animals such as rats and mice (Timmermans et al. 2019). In addition, GCs also regulate its own production, acting as negative feedback through MRs and GRs in various areas of the brain (Herman et al. 2012). As for receptor affinity, GCs have more for MR, occupying it at low concentrations such as at basal levels. On the other hand, the GR are occupied when the GCs are in high concentrations as during stress situations (Sorrells and Sapolsky 2007).

Different factors can activate the HPA axis, from immune responses to emotional stress, increasing the GCs release (Timmermans et al. 2019). Following exposure to a stressor, HPA axis is activated and GCs are released in the brain to restore physiological and behavioural homeostasis (Yiallouris et al. 2019). In addition to this adaptive response, the involvement of these hormones in neuronal activities such as nerve cell excitability, neuronal survival, neuroplasticity and neurogenesis is suggested (Oakley and Cidlowski 2011). In normal conditions, lower cortisol levels

interact with cortical and limbic structures, such as prefrontal cortex and hippocampus, to promote cognitive and emotional processing (Qin et al. 2016). However, at chronically elevated levels, GCs can have negative structural and functional consequences in these important brain structures (Sorrells and Sapolsky 2007; McEwen and Morrison 2013). Moreover, these limbic structures are also implicated in inhibition of the stress response due to their broad GR expression, which detects GCs at high levels and mediates the negative glucocorticoid feedback (Jankord and Herman 2009; Herman et al. 2012). Although the physiology of cortisol/corticosterone is already well understood, the interrelationships between stress, cortisol and depression remain not fully understood and this will be discussed in the next section.

HPA axis and depression

Altered HPA axis activity corresponds to one of the most consistent findings in the biology of depression, where 20-80% in depressed individuals present some form of HPA axis hyperactivation (Stetler and Miller 2011; Murri et al. 2014). Moreover, the depressive disorder often is related to hypercortisolism and this excessive GCs level can be characterized as a biological risk factor of this disease (Gibbons and Mchugh 1962; Cosgriff et al. 1990). In this way, the chronic exposure to corticosterone, main GC of rodents, is a widely used model for inducing depressive-like behaviour in rats and mice (David et al. 2009; Rainer et al. 2012). This pharmacological intervention mimics a stress situation, inducing changes in behaviour and immune system like those found in depressed patients (Ago et al. 2008; Valvassori et al. 2013). Other animal models of depression also suggest hyperactivation of the HPA axis, and some studies are shown in Table 1. Additionally, chronic administration of ACTH in rodents also presupposes the HPA axis involvement in antidepressant response (Kitamura et

al. 2002). This model induces an antidepressant-resistant phenotype through excessive activation of the HPA axis, inciting constant secretion and perpetuating elevated GC levels (Gaffey et al. 2016). After 14 days of ACTH administration, Srikumar et al. (2017) did not observe depressive-like behaviour development. Nevertheless, this treatment blocked the antidepressant effect of fluoxetine, imipramine and duloxetine. Moreover, this protocol also promoted an increase in adrenal gland weight and plasma corticosterone levels (Srikumar et al. 2017).

Evidence also suggests that stress, in addition to the hyperactivation of the HPA axis, may impair both its development and brain development (Mcewen 2000; Matthews 2002). In this context, the early life stress (ELS) experimental model, which can be accomplished through prenatal or neonatal stress, has been reported to reprogram the HPA axis, inducing HPA axis hyperactivity in adulthood (Bodegom et al. 2017). Rodent studies also suggest a causal relationship between ELS and endocrine and behavioural alterations similar to those found in depression (Kloet et al. 2005). These negatives consequences of early exposure to adverse events also are observed in humans. Childhood stress and trauma are factors that increase HPA axis activity and predispose to the development of psychopathologies (Harkness et al. 2006). In a cross-sectional study, depressive patients reported a higher occurrence of childhood stress than healthy controls. The most reported stressors were emotional and sexual abuse and family conflicts (Saleh et al. 2017). Moreover, Peng et al. (2014) observed higher HPA axis activity in depressed patients who suffered childhood neglect than depressed patients did not, revealing a positive correlation between ELS and HPA axis functioning (Peng et al. 2014).

Further HPA axis-related findings may be altered in depressive patients, and some studies are shown in Table 2. In this context, normalizing HPA axis activity may

be beneficial in treating depression (Soria et al. 2018). It has been demonstrated that an excessive GC signalling and hyperactivation of GR result in reduced neurogenesis and neuroplasticity, especially in the hippocampus, which is associated with functional impairments in the depressed brain (Anacker et al. 2011; Freitas et al. 2014). In this way, Ago et al. (2008) observed an antidepressant-like effect of a specific GR antagonist, RU-43044, in mice submitted to chronic corticosterone administration (Ago et al. 2008). However, in clinical studies, although improving symptoms of depression with psychotic characteristics, GR antagonist use in major depression is questionable. By blocking negative feedback via GR antagonism, mifepristone increases plasma ACTH and serum cortisol, which are usually already increased in depressed patients (Flores et al. 2006; Carroll et al. 2007). CRF receptor antagonism is also suggested as a possible alternative to improve depressive symptoms. Dong et al. (2018) found that treatment with CRF receptor antagonists R121919 and antalarmin prevented stress-induced behavioural changes in aged mice by reversing HPA dysfunction activity (Dong et al. 2018). Clinical evidence of the antidepressant effect of these antagonists has also been reported (Holsboer and Ising 2008). Treatment of depressive patients with R121919 was well tolerated and was able to reduce clinical scores for depression and anxiety (Zobel et al. 2000).

Another strategy that may be beneficial in mitigating the neurotoxic effect of GCs is the use of dehydroepiandrosterone (DHEA). This steroid is synthetized mainly by adrenal but also by gonads and brain and acts like an anti-glucocorticoid independently of GC receptors (Stárka et al. 2015). In Souza et al. (2018) review and meta-analysis, the effect of this compound on the improvement of depressive symptoms was more significant than placebo, suggesting its possible use in the treatment of depression. Finally, it is important to note that antidepressants already

available in the clinic improve symptoms of depression not only by improving neurogenesis but also by normalizing normal HPA axis activity and stress responsiveness (Surget et al. 2011; Mahar et al. 2014).

Microglia and HPA axis

As mentioned earlier, both microglia and HPA axis are related to depressive disorder. Moreover, an interaction between these immune cells and HPA axis is suggested, but has a partially unknown mechanism (Franchimont 2004; Coutinho and Chapman 2011). Microglia, a component of the innate immune system, is distributed within CNS in all tissues involved in the functioning of the HPA axis (O'Connor et al. 2000). In this context, oxidative and inflammatory mediators released after microglial activation may synergistically induce neuroinflammation and HPA axis activation (Colton 2009). Yet, all HPA axis components can secrete proinflammatory cytokines, once non-immune cells also can produce these mediators for the signal neuroimmune cells (Gadek-Michalska et al. 2013). Additionally, IL-1β is considered the main proinflammatory cytokine related to the regulation of the HPA axis (Goshen and Yirmiya 2009). This proinflammatory mediator can induce the CRF secretion in the hypothalamus and the ACTH in the pituitary, stimulating the GC production (Berkenbosch et al. 1987).

In this regard, Van der Meer et al. (1996) demonstrated that intravenous (iv) and intracerebroventricular (icv) administration of recombinant IL-1β and IL-6 activated HPA axis and increased corticosterone plasma levels in a dose-dependent manner. This increase was reverted by iv pre-treatment with recombinant human IL-1 receptor antagonist, demonstrating the regulatory action of IL-1β on the HPA axis (van der Meer et al. 1996). Also, 14 days of recombinant IL-1β icv injections in rats induced depressive behaviour, increased serum corticosterone levels and CD11b expression.

In this mentioned study, it was also seen that mifepristone, GR antagonist, reverted these changes suggesting microglia and HPA axis integration (Y.-P. Zhang et al. 2018).

Since microglia is the main source of inflammatory mediators in the CNS, Majidi et al. (2016) also investigated the effect of minocycline treatment during development of mice exposed to LPS, an immune response activator, in the neonatal period. Depressive- and anxiety-like behaviour, HPA axis activity and hippocampal inflammation in adulthood were evaluated. LPS induced behavioural changes, including increased serum corticosterone and IL-1β and TNF-α levels. However, minocycline was able to reverse behavioural changes, inflammatory and GC levels (Majidi-zolbanin et al. 2016). These results suggest that microglial activation by an immune challenge induces neuroinflammation and GC hypersecretion. Furthermore, under neuroinflammatory conditions can also be found high levels of GC due to HPA axis activation by proinflammatory cytokines (Munck et al. 1984).

Studies also have suggested the GCs as moderators of stress-related inflammation by modulating microglia in the CNS (Sorrells et al. 2009; Munhoz et al. 2010). Microglial cells correspond to the prime target of GCs in the CNS, expressing GR and MR (Sierra et al. 2008). Accordingly, Sugama et al. (2013) investigated the influence of corticosterone administration and rat adrenalectomy on stress-induced microglial activation. The results showed that the activation of these cells is markedly increased by adrenalectomy and suppressed by corticosterone treatment (Sugama et al. 2013).

Other study evaluated the short-term effects of adrenalectomy, demonstrating temporary increment on the levels of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α in adrenalectomized rats (Hamadi et al. 2016). Moreover, this study showed neuronal

cell death in the hippocampus succeeding adrenalectomy, suggesting that inflammation predates neurodegeneration. However, as well as the absence of GC by adrenalectomy, its excess may trigger a persistent sensitization of the microglia, keeping it in the inflammatory state (Ménard et al. 2017). It has been described that a persistent increase in GC may activate microglia, inducing neuroinflammation, suppression of neurotrophin production and neuronal apoptosis (Y.-P. Zhang et al. 2018). Interestingly, both GC increase and neuroinflammation have been reported as participants in the pathophysiology of depression.

Conclusion

As depression is a public health problem worldwide, the investigation of the routes involved, and their relationship is essential for a better understanding of the pathophysiology and treatment of this disorder. In this review we discussed the role of the microglia and the HPA axis, as well as the relationship between them and possible modulation for the treatment of depression. We believed that these immune and neuroendocrine systems work in a coordinated manner and that their dysregulation may be involved in the pathophysiology of major depression, since neuroinflammation and hypercortisolism are often observed in this disorder. Finally, additional studies involving experimental modulation and the evaluation of the integration of these pathways are interesting and necessary to clearly establish this connection.

Acknowledgements

This work was supported by scholarships from Coordination of Improvement of Higher Education Personnel (CAPES/Brazil) and from National Council for Scientific and Technological Development (CNPq/Brazil).

Statement of interest

No potential conflict of interest.

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Reference	Animal Specie	Protocol	Main findings
Athira et al., 2018	Male Swiss Mice	Chronic Corticosterone Administration	Development of depressive-like behaviour ↑ serum corticosterone ↑ serum ACTH
Li et al., 2018	Male Wistar Rats	Chronic Unpredictable Mild Stress (CUMS)	Development of depressive-like behaviour ↑ serum corticosterone ↑ serum ACTH
S. Wang et al., 2018	Male ICR Mice	Restraint Stress	Depressive-like behaviour and anxiety-like behaviour ↑ CRF expression ↑ CRF receptor expression ↑ serum corticosterone ↑ serum ACTH
Zheng et al., 2019	Male and Female Sprague-Dawley	Prenatal Stress	Depressive-like behaviour ↑ serum corticosterone ↑ serum ACTH

Table 1. Involvement of the HPA axis in experimental models of depression.

Reference	Diagnosis	Sex/Age range	Main findings
Stetler and Miller 2011	Major depression and subtypes	Men and women Young (under 18 years old) Old (over 65 years old) Undeclared	Tendency to ↑ cortisol levels ↑ ACTH levels No changes in CRH levels
Murri et al. 2014	Major depression	Men and women / equal or over 60 years old)	↑ cortisol levels in blood, saliva and urine
Iob et al. 2019	Depression	Men and women / 50 years old and over	↑ cortisol levels in hair and plasma
Pariante and Lightman 2008	Major depression	Undeclared	Hypertrophic pituitary / adrenal glands Hyperfunctioning pituitary / adrenal glands
Juruena 2014	Major depression history	Undeclared	Post-mortem: ↑ CRF mRNA levels Post-mortem: ↑ CRF-expressing neurons in the hippocampus ↓ CRF receptors in the pituitary

Table 2. Involvement of the HPA axis in depression.

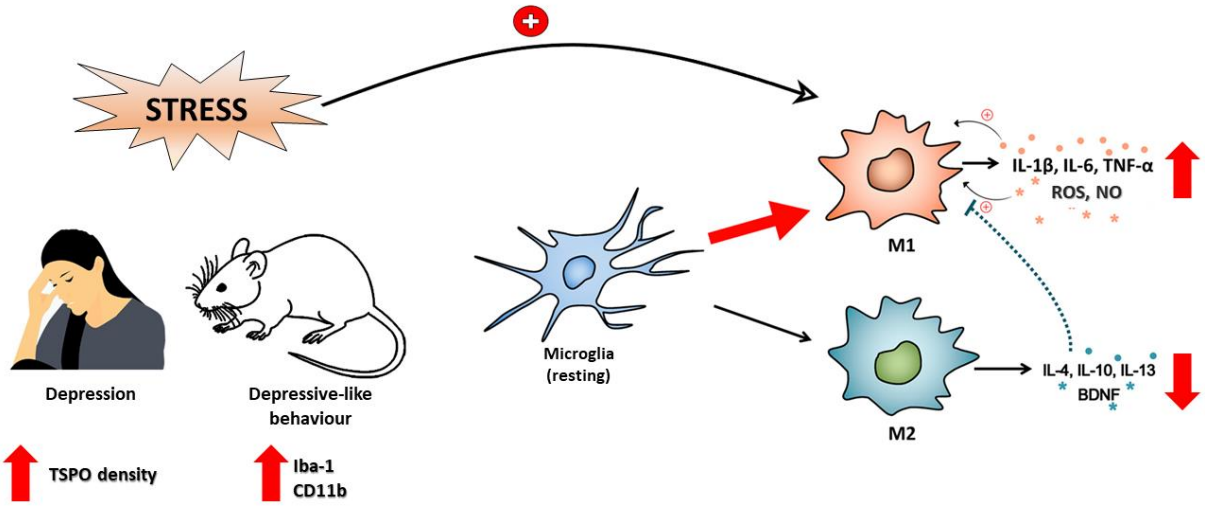

Figure 1. Involvement of microglia in depression. Stress, common factor in the development of depression and depressive-like behaviour, can activate the microglia, mainly its M1-phenotype and stimulate the secretion and release of pro-inflammatory (IL-6, IL-1β and TNF-α) and oxidative (ROS and NO) mediators. Consequently, it is common to find an excess of these mediators in depressed patients and rodents with depressive-like behaviour. Reduction in anti-inflammatory cytokines (IL-4 and IL-10) and neurotrophic factors (BDNF) is also found in these conditions. In addition, another finding that suggest the participation of microglia in depression is an increase in the density of TSPO protein, as well as an increase in the expression of Iba1 and CD11b markers in the brain of rodents that exhibit depressive-like behaviour.

3.2. MANUSCRITO 2: Glucocorticoid and brain-derived neurotrophic factor relationship: a brief investigation into the model of depression by chronic administration of corticosterone

O manuscrito apresentado a seguir inclui as seções de “Introdução”, “Materiais e Métodos”, “Resultados e Discussão” e “Referências”. O formato apresentado é o que foi aceito para publicação no periódico *Behavioural Pharmacology*.

Glucocorticoid and brain-derived neurotrophic factor relationship: a brief investigation into the model of depression by chronic administration of corticosterone

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Conflicts of interest: none

Source of funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. This work was supported by scholarships from Coordination of Improvement of Higher Education Personnel (CAPES/Brazil).

Abstract

Depression is considered a common mental disorder that affects more than 300 million people worldwide (WHO, 2017). Despite this high incidence, its etiology is not completely elucidated instigating further studies. For this purpose, different animal models are used to study routes and molecular changes involved in depression, among them the chronic administration of corticosterone. However, the knowledge about neurochemical changes after this protocol is still controversial. In this work, we evaluated serum corticosterone levels, adrenal/body weight ratio, as well as glucocorticoid receptor (GR) and brain-derived neurotrophic factor protein expression and its receptor, tropomyosin receptor kinase B. These analyzes were performed on prefrontal cortex, hippocampus, and striatum samples taken of mice after 21 days of administration of corticosterone. Exposure to corticosterone reduced the serum corticosterone levels and the adrenal/body weight ratio. Moreover, the GR and TrkB expression were increased in the hippocampus while the BDNF expression was reduced in the prefrontal cortex. We also found a positive correlation between the expression of glucocorticoid receptor and tropomyosin receptor kinase B and our results suggest a possible relationship between the glucocorticoid / glucocorticoid receptor and brain-derived neurotrophic factor / tropomyosin receptor kinase B routes after chronic corticosterone administration. To our knowledge, this is the first study that evaluate these parameters concomitantly in important mood-related structures. In addition, these results may be useful to other research groups seeking to explore new pathways and substances with therapeutic potential to treat this silent epidemic.

Keywords: adrenal / body weight ratio, brain-derived neurotrophic factor, depression model, glucocorticoid receptor, serum corticosterone levels, tropomyosin receptor kinase B.

1. Introduction

Depression is considered a common mental disorder that affects more than 300 million people worldwide (WHO, 2017). Despite this high incidence, the etiology of this silent epidemic is not completely elucidated, making treatment difficult. This motivates the study of depression physiopathology in attempt to find new alternatives for antidepressant therapy since there is a large proportion of patients resistant to all available treatments (Akil et al., 2018). For this purpose, different animal models are used to study routes and molecular changes involved in depression.

Several animal models are based on exposure to some stress, among them the chronic administration of corticosterone, the main GC hormone of rodents. This is a pharmacology intervention that produces similar changes in the behavior and immune system which are found in patients with depression (Valvassori et al., 2013). Furthermore, the depression has been associated with abnormalities in neurotransmitter expression, structural changes in the brain, as well as in the hypothalamus-pituitary-adrenal axis (HPA axis) (Patel, 2013).

The disruption of neurogenesis and alterations on neurotrophic factors levels, including brain-derived neurotrophic factor (BDNF), are also associated with depressive behavior (Autry and Monteggia, 2012). BDNF and its receptor, TrkB, are abundantly expressed in the neurons of the central nervous system (CNS), where they contribute to neuronal survival, synaptogenesis, and plasticity (Björkholm and Monteggia, 2016). Depressive patients often exhibit lower levels of BDNF, as well as its reduced TrkB receptor signaling (Yi et al., 2014; Castrén and Kojima, 2017). These alterations are usually observed in cerebral structures such as prefrontal cortex, hippocampus, and striatum, which are areas related to emotional and cognitive processes supposedly involved in depressive disorder (Pandya et al., 2012). For this

reason, these are the most commonly used structures in the research of neurological disease such as depressive disorder (Dusi et al., 2015). Also, abnormalities in GC and its receptor, GR, are also suggested as participants in depressive behavior (Anacker et al., 2011). However, the knowledge about the changes mentioned above after chronic corticosterone administration is still controversial.

Therefore, this study aimed to investigate the expression of GR, BDNF, and TrKB in prefrontal cortex, hippocampus, and striatum in mice submitted to chronic administration of corticosterone. In addition, we evaluated the development of depressive-like behavior by the tail suspension test, the corticosterone serum levels as well as the adrenal/body weight ratio after corticosterone administration for 21 days in mice.

2. Methods

2.1. Drugs

Corticosterone (Sigma-Aldrich, St. Louis, Missouri, USA) was dissolved in hypotonic saline solution with 0,2% Tween 80 and 0,2% dimethyl sulfoxide (DMSO) at dose of 20 mg/kg/10 ml for the subcutaneous administration (Bai et al., 2018; Parente et al., 2018). The vehicle used was hypotonic saline solution (0.9% NaCl with 0,2% Tween 80 and 0,2% DMSO) without corticosterone.

2.2. Animals and experimental procedure

Male Swiss mice (20–35 g) were kept at 20–25ºC with free access to food and water, under a 12:12 h light/dark cycle, and were submitted to one week of acclimatization. The animals were divided into two groups: control, that received vehicle subcutaneously, or stressed, that received corticosterone, once a day for 21 days. Twenty-four hours after the end of induction protocol, the animals were submitted

to tail suspension test and after were anesthetized and cardiac puncture was performed for blood collection. In the sequence, mice were euthanized, prefrontal cortex, hippocampus, and striatum were removed and stored frozen until analysis. All the experiments were performed in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and approved by our Institutional Ethics Committee (Process number 7698200617/2017). Furthermore, all efforts were made to minimize animal suffering, as well as we use only the number strictly necessary to carry out the analyzes. The experiment timeline is shown in Fig. 1A.

2.3. Tail suspension test

This test was used to assess the development of depressive-like behavior and was conducted according to Steru et al. (1985) with adaptations. For this, the animals were suspended by the tail 50 cm above the floor for six minutes and the immobility time measured, in seconds, during the last four minutes. The observers who performed this test were blinded to the experimental groups.

2.4. Corticosterone serum levels

The serum was obtained after whole blood centrifugation at 3000 xg at room temperature for 10 min and stored until analysis. The corticosterone circulating level was measured employing an Enzyme-Linked Immunosorbent Assay (ELISA) Kit (Enzo Life Sciences, Farmingdale, NY, EUA) following the product manual. Briefly, the samples were diluted according to corticosterone ELISA small volume protocol for serum/plasma. Then, 100 µL of sample or standard solutions were incubated at the plate with the antibody provided by the kit at room temperature on a plate shaker for 2

h. After, the wells were empty and washed and added 200 µL of pNpp Substrate Solution incubating for 1 h without shaking. The reaction was stopped with 50 µL of stop solution, and the plate was read at 405 nm using a microplate reader (SpectraMax I3, Molecular Devices, San Jose, California, USA).

2.5. Adrenal/body weight ratio

To evaluate the possible relationship between adrenal and body weight, the animals were weighed at the end of the induction protocol. In euthanasia, the adrenal gland was removed and weighed. The adrenal/body weight ratio was calculated by dividing adrenal weight by body weight, and the result was expressed in mg/g.

2.6. Western blotting assay

Prefrontal cortex, hippocampus and striatum samples were homogenized with a lysis buffer containing 137 mM NaCl, 20 mM Tris–HCl pH 8.0, 1% NP40, 10% glycerol, 1 mM phenylmethylsulfonylfluoride (PMSF), 10 µg.mL−1 aprotinin, 0.1 mM benzethonium chloride, and 0.5 mM sodium vanadate. Homogenates were then centrifuged (11400 rpm per 30 min). Then, the supernatant was collected, and total protein concentration was determined according to the Lowry method) using bovine serum albumin (BSA) as standard (Lowry et al., 1951). After, protein samples were separated by electrophoresis on a 10% or 12,5% polyacrylamide gel and electrotransferred to a PVDF membrane (Millipore, Burlington, Massachusetts, USA). Non-specific binding sites were blocked in Tris-buffered saline (TBS), pH 7.6, containing 5% non-fat dry milk. Membranes were rinsed in buffer (0.05% Tween-20 in TBS) and incubated with primary antibodies: anti-β-actin (1:50000; Sigma-Aldrich), anti-BDNF (1:2000; Abcam, Cambridge, UK), anti-TrkB (1:500; Santa Cruz

Biotechnology, Dallas, Texas, USA) and anti-GR (1:500; Santa Cruz Biotechnology), followed by anti-rabbit (1:20000; Santa Cruz Biotechnology) IgG horseradish peroxidase conjugate. Immunocomplexes were visualized using Luminata (Millipore) according to the manufacturer's instructions. Film signals were digitally scanned (Chemidoc^{TM} Imaging Systems, Bio-Rad, Hercules, California, USA) and then quantified using ImageJ software. β-actin was used as an internal control and each data was normalized according to its own actin value. Data were expressed as % of control, where the values were calculated considering the average of the normalized values of the control group as 100%.

2.7. Statistical analyses

All data are expressed as mean ± SEM and were analyzed using GraphPad Prism (version 7.00, San Diego, USA). The differences between groups were determined by t test (parametric data), and the Pearson correlation was performed to evaluate the correlation between GR expression and BDNF and TrkB expression. Results were considered significant at $P < 0.05$.

3. Results and Discussion

First, to verify the development of depressive-like behavior, the mice were submitted to tail suspension test. The stressed group demonstrated higher immobility when compared with control group [Fig. 1B, $t(18) = 2.137$, $P < 0.05$] indicating the development of depressive-like behavior. As shown in Fig. 1C, chronic corticosterone treatment reduced the corticosterone serum levels [$t(8) = 4.149$, $P < 0.05$]. According to this, we also observe that the adrenal/body weight ratio was lower in the stressed group [Fig. 1D, $t(8) = 8.869$, $P < 0.05$]. These results suggest the hypofunction of the

adrenal gland and can be associated with the interference of exogenous glucocorticoid administered. In this way, the functioning of the HPA axis may be deregulated, and the endogenous production of corticosterone suppressed. Although some studies report the opposite, other literature data show similar results with reduction of corticosterone serum levels in animals that received corticosterone solution for 21 days (Pazini et al., 2016; Rosa et al., 2014). Together, these data suggest that changes in GCs levels can be closely related to the development of depression or depressive-like behavior. Agreeing with that, antidepressant treatment usually normalizes plasma GCs levels, and, when it does not, patients have an increased risk of relapse (Holsboer and Ising, 2010).

The mice that received corticosterone administration also had an increase in GR protein expression in the hippocampus [Fig. 2E, $t(4) = 3.156$, $P < 0.05$] without significant increase in prefrontal cortex [Fig. 2A, $t(5) = 2.125$, $P = 0.09$] and striatum [Fig. 2I, $t(6) = 0.181$, $P = 0.86$]. The GRs are widely expressed in CNS, including structures related to the typical brain stress circuit such as the hypothalamus, hippocampus, and amygdala (De Kloet, 2013). These receptors correspond to the main target of GCs in stressful conditions, and its function is to suppress stress and immune response (Miller et al., 1998). However, the role of this receptor in pathologies such as depression is contradictory. We found a GR upregulation that may be assumed as a positive marker of corticosterone action, which in the long-term exerts neurotoxic effects (Kurek et al., 2016). These effects include impaired behavior and neurogenesis, reduced survival and neuronal plasticity, and morphological changes, stimulating the development of depressive behavior (Kim and Diamond, 2002; Anacker et al., 2013; Jeanneteau and Chao, 2013). According to this, Woo et al. (2018) observed an increase in GR hippocampal expression after the chronic restraint stress protocol and

in hippocampal neurons of mice exposed to corticosterone. Likewise, Wei et al. (2004) reported that the GR overexpression in the forebrain induces an increase in emotional instability. The opposite situation was also seen by Tronche et al. (1999), where GR-deficient mice exhibited a reduction of anxiety- and depressive-like behavior.

In addition to neuronal damages cited above, mood disorders such as depression may also exhibit changes in structure and synaptic transmission. These events are regulated by BDNF, an important neurotrophin with various CNS functions (Caviedes et al., 2017). We found the protein expression of BDNF reduced in the prefrontal cortex of the stressed group [Fig. 2B, $t(4) = 2.632$, $P < 0.05$] without change in the immunocontent of its receptor [Fig. 2C, $t(4) = 0.198$, $P = 0.85$]. On the other hand, in the hippocampus was observed an upregulation of TrkB receptor [Fig. 2G, $t(6) = 2.613$, $P < 0.05$] without change in the protein expression of BDNF [Fig. 2F, $t(4) = 1.712$, $P = 0.16$]. In the striatum, no differences were observed in the immunocontent of BDNF [Fig. 2J, $t(6) = 0.430$, $P = 0.68$] and its receptor [Fig. 2K, $t(6) = 1.651$, $P = 0.15$]. BDNF/TrkB pathway in depression is reported as complex and dependent on the brain region (Boulle et al., 2012). Reduced levels of this neurotrophin in prefrontal cortex and hippocampus are related to atrophy and cell loss and are commonly found in depressed patients (Duman and Monteggia, 2006; Martinowich et al., 2007; Zhang et al., 2016). However, increased BDNF/TrkB pathway activity in reward system-related brain has been associated with the development of depressive- and anxiety-like behavior (Krishnan et al., 2007; Boulle et al., 2012). In this context, Cazorla et al. (2011) demonstrated that systemic administration of a TrkB antagonist in mice has an antidepressant and anxiolytic effect, decreasing TrkB brain activity without affecting neuronal survival. In our study, we found a reduction in BDNF immunocontent accompanied by increased TrkB protein expression. This TrkB receptor alteration may

be an adaptive response to restore signaling of this pathway, impaired by chronic corticosterone administration.

We also found a positive correlation between GR and TrkB protein expression in the hippocampus (Fig. 2H, $r = 0.85$, $P < 0.05$) and striatum (Fig. 2L, $r = 0.87$, $P < 0.05$), reinforcing the possible interaction of this pathways. Moreover, the neurotrophic hypothesis of depression proposes that the reduction of BDNF mediates the neurotoxic effects of prolonged GR activation mentioned above (Duman and Monteggia, 2006). Other authors and we believe that GR and BDNF activity works in a regulated way (Jeanneteau and Chao, 2013). It has also been proposed that BDNF may function as a conditioning factor of the response to GCs as well as its deregulation may compromise the responses coordinated by the GR, suggesting a GC and BDNF relationship (Jeanneteau et al., 2019).

Barfield and Gourley (2018) gathered a collection of data relating cortical prefrontal TrkB, glucocorticoids and their interactions in stress situations. However, to our knowledge, our study is the first that concomitantly evaluates three important structures (prefrontal cortex, hippocampus and striatum) related to mood disorders, following the chronic corticosterone administration protocol.

In conclusion, our study suggests a possible relationship between GC / GR and BDNF / TrkB routes after chronic corticosterone administration. This is a step to elucidate the pathophysiology of depression that may be helpful to other research groups studying depression and alternatives to its treatment. As a perspective, it is necessary to intensify studies and test the modulation of these pathways using new or known substances, but with possible new therapeutic potential.

Acknowledgments

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. This work was supported by scholarships from Coordination of Improvement of Higher Education Personnel (CAPES/Brazil).

Conflict of interest

There are no conflicts of interest.

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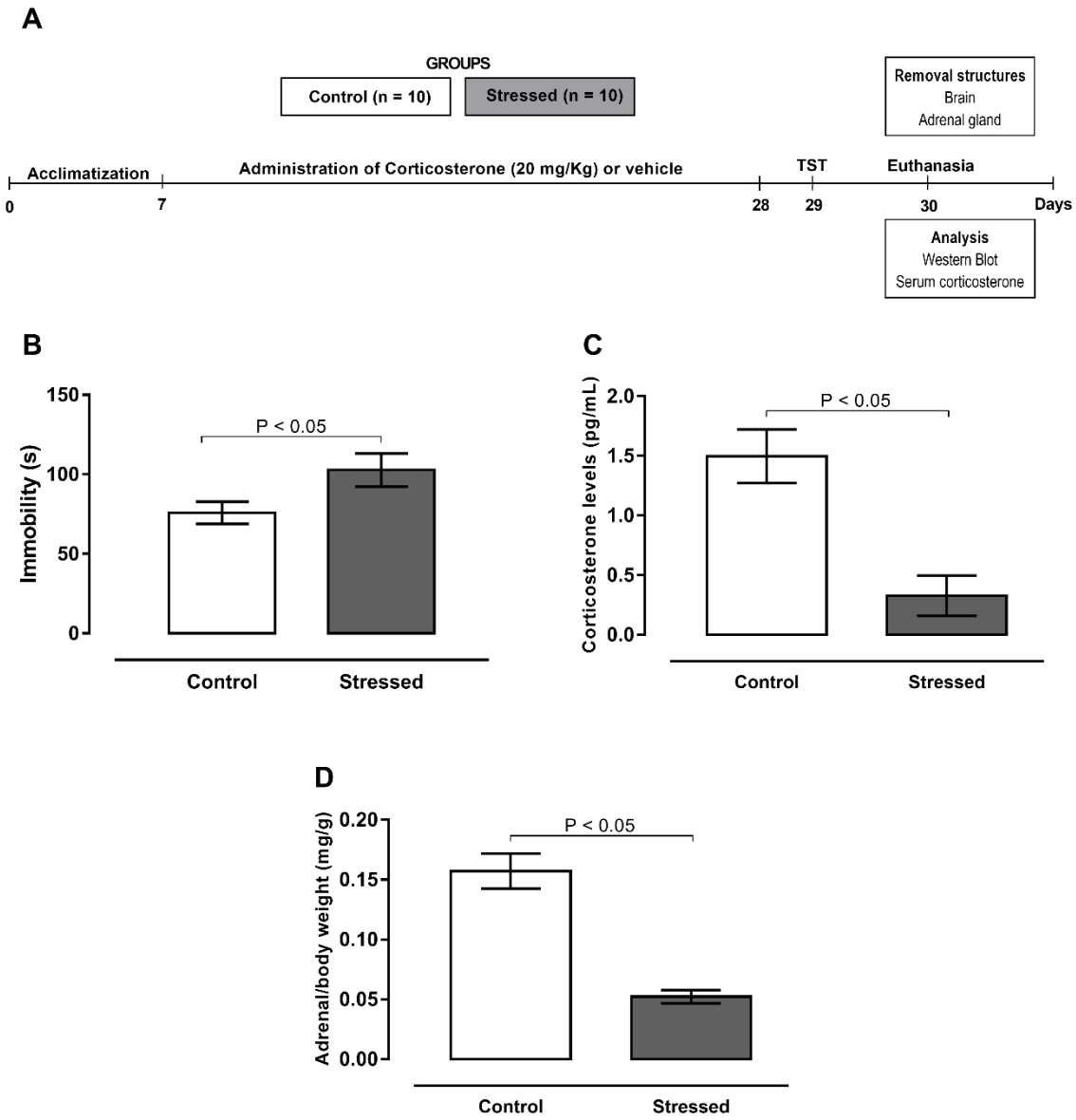
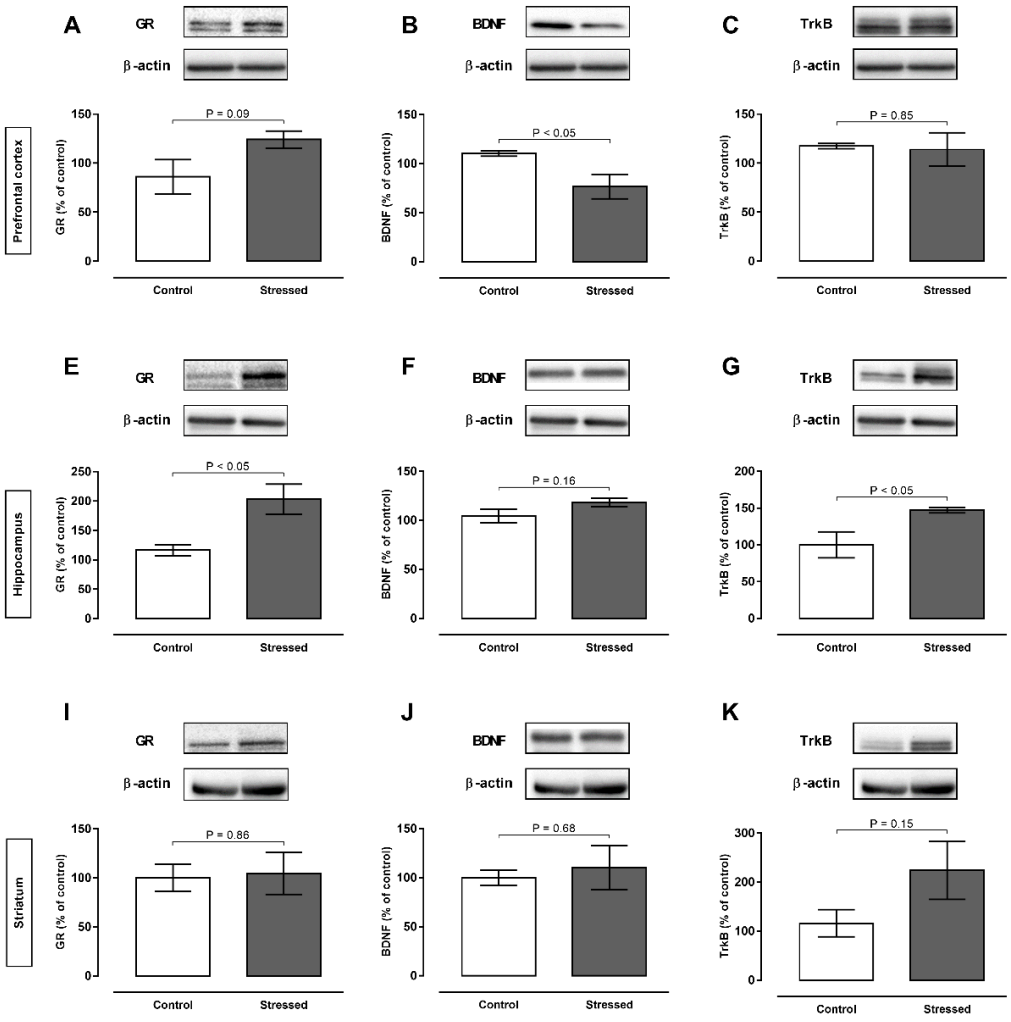


Figure 1 – Chronic corticosterone administration protocol. (a) The experimental design. (b) Tail suspension test (n = 10). (c) Serum levels of corticosterone expressed in logarithmic form (n = 5). (d) Adrenal/body weight ratio (n = 6).



D

GR	Pearson r	P value
BDNF	0.32	0.47
TrkB	0.62	0.13

H

GR	Pearson r	P value
BDNF	0.62	0.14
TrkB	0.86	0.02*

L

GR	Pearson r	P value
BDNF	0.74	0.06
TrkB	0.87	0.01*

Figure 2 – Effects of chronic corticosterone administration on GR, BDNF and TrkB protein expression in the prefrontal cortex (a–c) hippocampus (e–g) and striatum (i–k) (n = 3–4). Correlations between GR and BDNF and GR and TrkB in the prefrontal cortex (d) hippocampus (h) and striatum (l) (n = 3–4). BDNF, brain-derived neurotrophic factor; GR, glucocorticoid receptor; TrkB, tropomyosin receptor kinase B.

3.3. MANUSCRITO 3: Apocynin as an antidepressant agent: *in vivo* behavior and oxidative parameters modulation

O manuscrito apresentado a seguir apresenta as seções “Introdução”, “Materiais e Métodos”, “Resultados”, “Discussão”, “Conclusão” e “Referências”. O formato apresentado segue as normas do periódico *Behavioural Brain Research*, para o qual foi submetido.

Apocynin as an antidepressant agent: in vivo behavior and oxidative parameters modulation

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Declarations of interest: none.

Abstract

Depression is one of the most common mood disorders, which affects one in six people at some point in life. However, the treatment of this disease is still a challenge. Chronic corticosterone administration (CCA) is a widely used animal model to study the mechanisms involved as well possible therapeutic strategies for treatment of depression. Moreover, elevated oxidative stress has been observed in psychiatric disorders including major depression and, in this context, antioxidant therapy may be a potential therapeutic alternative. In this study, we investigated the effect of seven days of treatment with apocynin, an antioxidant of natural origin, on depressive-like behavior and oxidative parameters in mice submitted to CCA. After 21 days of corticosterone administration (20 mg/Kg/day, subcutaneously, s.c.), we observed the development of depressive-like behavior with increase in immobility time on tail suspension test and on forced swimming test, and reduction in total grooming time on splash test. Moreover, we found high superoxide dismutase activity as well as hydrogen peroxide levels whereas catalase activity was reduced in the prefrontal cortex, hippocampus and striatum of mice. Seven days of treatment with apocynin (100 mg/Kg/day orally, p.o), performed immediately after corticosterone administration in the last week of protocol, was able to reverse the most of these changes, revealing its antidepressant-like effect. In conclusion, our results suggest apocynin as an antidepressant-like agent with a mechanism of action based on the attenuation of oxidative changes induced by CCA.

Keywords: Depression; Corticosterone; Stress; Antioxidant; ROS.

1. Introduction

Major depression is one of the most common mood disorders, which affects one in six people at some point in life [1]. Depressed patients may have depressed mood condition, irritability, cognitive impairment, and sleep and appetite disorders [2,3]. Because it is a complex multifactorial disease and its pathophysiology has not yet been elucidated, the treatment of depression is still a challenge [4,5]. In this context, animal models of this disease have been used to investigate possible mechanisms as well as new therapeutic strategies. Among them there is the chronic corticosterone administration (CCA) model. Corticosterone is the main glucocorticoid hormone of rodents, that induce the depressive-like behavior in rats and mice producing changes similarly to those found in patients with depression [6,7]. Also, increased oxidative stress has been reported in both animal model and depressive disorder in humans [8].

Oxidative stress is a complex process characterized by a persistent imbalance between free radical or nonradical molecules synthesis and their degradation by antioxidant mechanisms [9]. The oxidative agents involved in this event include both reactive oxygen species (ROS) and reactive nitrogen species (RNS) [10]. ROS comprise radical such as superoxide anion ($O_2^{\bullet -}$) and hydroxyl ($^{\bullet}OH$), as well as non-radical species such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and singlet oxygen ($^1O_2$). On the other hand, RNS include nitric oxide (NO), peroxynitrite ($ONOO^{\bullet}$) and nitrogen dioxide (NO_2) [11].

These species participate in cellular signaling and homeostasis, however their overproduction can damage cellular components and compromise the functionality of many cells [12]. Moreover, the central nervous system (CNS) is quite susceptible to the deleterious effects of oxidative stress, which can negatively impact the brain and normal CNS functions [13]. Growing evidence suggests the participation of the enzyme

complex nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) as an important source of ROS in the brain [14]. It consists in a complex structure, including the membrane-bound catalytic subunit NOX, which may be presented as different isoforms [15]. NADPH oxidase is broadly expressed in neurons and utilizes NADPH and oxygen to produce $O_2^{\bullet -}$, which is quickly dismutated by superoxide dismutase (SOD) to H_2O_2, that in turn can be decomposed by catalase (CAT) or by glutathione peroxidase (GPx) [10,16].

The exact mechanisms by which oxidative stress relates to depressive symptoms are not yet clear. However, the extensive use of oxygen with the subsequent formation of reactive species makes the brain extremely vulnerable to oxidative damage [17]. These oxidative products can promote neurodegeneration and impair the function of structures such as prefrontal cortex, hippocampal and striatum, compromising the control of cognitive and mood functions, which are often impaired in depressed patients [18]. In addition, evidence also suggests that mitochondrial dysfunction may be involved in major depression [19]. Mitochondria is the main intracellular organelle that produces ROS and studies have already shown that prolonged exposure to glucocorticoids can cause abnormalities in its structure and favor the increase in ROS and its negative consequences [20–22]. Moreover, depressive disorder has also been characterized by reduced antioxidant status, which was improved after treatment with antidepressants such as fluoxetine, amitriptyline, nortriptyline and fluvoxamine [23,24]. In this context, compounds capable of prevent the formation or suppress the reaction between substrate and oxidizing species have been investigated for their possible neuroprotective and antidepressant effect [25].

Apocynin (4’-hydroxy-3’-methoxyacetophenone, also known as acetovanillone) is a compound originally isolated from the roots of the medicinal plant *Picrorhiza kurroa*

[26]. Similar to other phenolic compounds, apocynin has antioxidant activity acting as an inhibitor of NADPH oxidase [27]. Moreover, other evidence also suggests that it has anticancer and anti-inflammatory effects and prevents mitochondrial dysfunction [28–30]. Although somewhat limited, safety data for apocynin characterize it as low toxic and very stable [31]. Additionally, this compound is active by oral administration, making it a good candidate for future studies looking forward to new alternatives for treating inflammatory and oxidative conditions such as depression [32]. This becomes even more interesting considering the wide range of possible adverse effects of conventional antidepressants [33,34]. Thus, the objective of this work was to evaluate the antidepressant-like effect of apocynin in mice submitted to CCA evaluating the depressive-like behavior and oxidative profile of brain structures.

2. Material and methods

2.1 Animals

Forty-eight Swiss male mice (20–30 g; 4–6-week-old) obtained from the Federal University of Santa Maria breeding colony were maintained at 22 ± 2ºC, under 12h light-dark cycle and with free access to food and water. The animals were submitted to one week of adaptation to the new environment before the experiment. All experiments were performed according to the National Institute of Health Guide for Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and approved by our Institutional Ethics Committee (Process number 7698200617/2017). The number of animals used in this study was the minimum necessary to demonstrate consistent effects and every effort was made to minimize their suffering.

2.2 Chemicals and reagents preparation

When unspecified, the reagents used were from Sigma-Aldrich (St. Louis, MO). Corticosterone (Sigma-Aldrich Catalog Number C2505, Lote Number SLBT2966) was dissolved in hypotonic saline solution with 0,2% of Tween 80 and 0,2% of dimethyl sulfoxide (DMSO) at dose of 20 mg/Kg (solution concentration: 2 mg/mL) for the subcutaneous administration (s.c.) [6,35–37]. The vehicle used was a hypotonic saline solution containing 0,2% of Tween 80 and 0,2% of DMSO but without corticosterone. For the oral treatment, apocynin (Sigma-Aldrich Catalog Number W508454, Lote Number MKBP9013V) was diluted in hypotonic saline solution with 1% of DMSO and administered orally (oral gavage, p.o.) at dose of 100 mg/Kg (solution concentration:10 mg/mL) [38,39]. The vehicle was a hypotonic saline solution with 1% of DMSO but without apocynin, which was administered for the same route and period.

2.3 Experimental design

This study was divided into two phases (Figure 1). In phase 1, we induced the depressive-like behavior by the CCA and evaluated the behavior and oxidative parameters after this protocol. For this, the mice were segregated in two groups (n = 8 animals/ group): Vehicle (VEHICLE) or Corticosterone (CORT), that received subcutaneously hypotonic saline solution or corticosterone, respectively. The administrations were performed once a day between 8:00 AM and 11:00 AM for 21 consecutive days. Twenty-four hours after the last administration of vehicle or corticosterone the animals were submitted to behavioral tests. Twenty-four hours after, on day 30, the animals were anesthetized, euthanized by cervical dislocation and the brain structures (prefrontal cortex, hippocampus and striatum) were collected and stored for biochemical analysis. The timeline of this experiment is shown in Figure 2A.

Second in phase 2, after observing the changes in the phase 1, we investigated the effect of apocynin treatment on these parameters. Thus, the animals were submitted to the same induction protocol with corticosterone but, from the fifteenth day, were treated with apocynin (100 mg/Kg) or vehicle orally (gavage, p.o.), once a day immediately after the corticosterone injection, totalizing 7 days of treatment [38,39]. Twenty-four hours after the last corticosterone administration and apocynin or vehicle treatment, the animals were submitted to behavioral tests. Twenty-four hours after, on day 30, the animals were anesthetized and euthanized by cervical dislocation for the collection of the brain structures, which were stored frozen at -20ºC until biochemical analysis. The timeline of this experiment is shown in Figure 2B. In this phase we obtained the groups Corticosterone + Vehicle (CORT+VEH) and Corticosterone + Apocynin (CORT+APO) (n = 8 animals/group).

Additionally, in the phase 2, we evaluated the effect of apocynin in mice not exposed to corticosterone to investigate its *per se* effect. For this, mice were segregated in two groups: Vehicle + Vehicle (VEH+VEH) and Vehicle + Apocynin (VEH+APO) (n=8 animals/group), which received subcutaneous vehicle administrations for 21 days. The treatment with Apocynin (100 mg/Kg) or vehicle orally (gavage, p.o.) was started on the fifteenth day, once a day, immediately after the vehicle injection, totalizing 7 days of treatment. Twenty-four hours after the last vehicle administration and apocynin or vehicle treatment, the animals were submitted to behavioral tests. In the next day, on day 30, mice were anesthetized and euthanized by cervical dislocation for the collection of the brain structures, which were stored frozen at -20ºC until biochemical analysis. The timeline of this experiment is shown in Figure 2C.

2.4 Behavioral Tests

2.4.1 Tail Suspension Test and Forced Swimming Test

We used the tail suspension test (TST) and the forced swimming test (FST) to assess the depressive-like behavior. The TST was performed according to Steru et al. (1985), where the mice were suspended by the tail with an adhesive tape on an apparatus 50 cm above the surface of a table [40]. The test was performed for 6 minutes and the immobility time timed, in seconds, in the last 4 minutes [41]. The FST was performed as previously described by Porsolt et al. (1977) [42]. Mice were individually placed in a plastic container with dimensions that could not touch the bottom of the apparatus, either with the tail or feet, containing 30 cm of water at 25 ± 2ºC. Immobility was considered when the animal kept floating passively just to keep its nose out of the water and was counted for 6 minutes [5]. In both tests, increased immobility time suggests the development of depressive behavior [43].

2.4.2 Splash Test

The splash test (SPT) evaluates the animal self-care behavior by indirectly measuring the consumption of palatable solution [44]. For this, mice were placed individually in a transparent acrylic box and a 10% sucrose solution was squirted on the dorsal coat of animals. Immediately, the animal total grooming behavior was observed and timed for a period of 5 minutes. A decrease in grooming time is associated with anhedonia and depressive-like behavior [45].

2.4.3 Open-Field Test

The open field test (OFT) was performed according to Prut and Belzung (2003) and evaluated the locomotor activity of the animals [46]. For this, the animals were

placed in the center of a circular arena (30 x 30 x 15 cm) with the floor divided into equal quadrants where were evaluated the number of crossings (horizontal locomotion) and rearings (vertical activity) for 5 minutes.

2.5 Biochemical analyses

2.5.1 Protein levels

Before the biochemical analyses, we measured the protein levels in the samples. Prefrontal cortex, hippocampus and striatum samples were homogenized in 50mM Tris-HCl pH 7,4, centrifuged at 3000 rpm for 20 min at 4ºC and the supernatant collected. The method used for this determination in the supernatant was the Bradford method [47]. Supernatants were kept frozen at -20ºC until further biochemical analysis.

2.5.2 SOD-like activity, H_2O_2 levels and CAT enzyme activity

These analyses were performed on the supernatants of pre-frontal cortex, hippocampus and striatum samples. SOD-like activity was assessed according to Oliveira et al (2010) by the inhibition of superoxide anion-dependent adrenaline auto-oxidation to adrenochrome, a yellow chromophore [48]. Samples were incubated with sodium carbonate buffer (Na_2CO_3, 50 mM, pH 10.2) at 30ºC and the reaction was initiated by the addition of adrenaline (or epinephrine, 60 mM, pH 2.0). The SOD-like activity was determined spectrophotometrically at 480 nm (kinetic reaction for 2 minutes). The results were calculated and expressed as international units and corrected by protein content of the sample analyzed (UI SOD/mg protein). For the H_2O_2 determination, we used the phenol red-horseradish peroxidase method [49,50]. Briefly, the supernatant of samples was centrifuged with sodium azide 5mM (proportion 1:5) at 12000 g for 20 min at 4ºC. After, these supernatants were incubated with phenol red

(100 µg/mL) and horseradish peroxidase (1260 µg/mL) solutions at 25ºC for 10 minutes. At the end of incubation, sodium hydroxide (NaOH, 1M) was added to stop the reaction and then the absorbance was determined spectrophotometrically at 610 nm. The H_2O_2 levels were calculated based on standard curve of peroxidase-mediated oxidation of phenol red by H_2O_2 and expressed as µmol adjusted by tissue weight of sample analyzed (µmol H_2O_2/g tissue). For the CAT activity evaluation, we used a UV spectrophotometric method previously described by Aebi (1984) [51]. In this assay, supernatants of tissue samples were exposed to H_2O_2 solution (0,3M) and the changes in the absorbance were monitored spectrophotometrically at 240 nm for 2 minutes. The results were calculated and expressed as µmol of CAT adjusted by protein content (µmol CAT/mg protein).

2.6. Statistical analysis

Data are expressed as mean ± SEM and were analyzed using GraphPad Prism (version 7.00, San Diego, USA). Previously, the values were tested by the Shapiro-Wilk normality test and then the Student's *t*-test was performed to evaluate the differences between groups.

3. Results

3.1 Depressive-like behavior induction and involvement of oxidative stress

Firstly, we investigated the depressive-like behavior of the animals after the induction protocol (phase 1). The animals submitted to CCA showed higher immobility time when compared with vehicle group in the FST [Fig. 3A, $t(14) = 2.367$, $P < 0.05$] and TST [Fig. 3B, $t(14) = 8.112$, $P < 0.0001$]. We also evaluated the behavior of motivation and self-care by the SPT, and the CORT group showed lower total grooming

time [Fig. 3C, t(14) = 3.148, P < 0.01]. In addition, no significant differences were found in locomotor activity evaluated in the OFT between vehicle and CORT group (crossing and rearing, Fig. 3D).

We also observed an increase in SOD unit in prefrontal cortex [Fig. 4A, t(7) = 5.637, P < 0.001], hippocampus [Fig. 4D, t(7) = 2.605, P < 0.05] and striatum [Fig. 4G, t(7) = 4.604, P < 0.01] of animal samples from CORT group. The H_2O_2 levels were also higher in prefrontal cortex [Fig. 4B, t(9) = 2.476, P < 0.05], hippocampus [Fig. 4E, t(9) = 2.623, P < 0.05] and striatum [Fig. 4H, t(9) = 2.713, P < 0.05] in the CORT group than in vehicle group samples. Additionally, a lower CAT activity was observed in the prefrontal cortex [Fig. 4C, t(10) = 3.056, P < 0.05], hippocampus [Fig. 4F, t(10) = 3.838, P < 0.01] and striatum [Fig. 4I, t(10) = 2.939, P < 0.05] of the CORT group samples.

3.2. Effect of apocynin on depressive-like behavior and oxidative parameters

In the phase 2, we tested the effect of apocynin on depressive-like behavior induced by CCA. Seven-day apocynin treatment was able to reverse the increased immobility caused by the induction protocol in both FST [Fig. 5A, t(14) = 5.396, P < 0.0001] and TST [Fig. 5B, t(14) = 7.055, P < 0.0001]. Apocynin also enhanced the grooming behavior in the SPT [Fig. 5C, t(14) = 5.032, P < 0.001] and did not alter locomotor activity in the OFT (crossing and rearing, Fig. 5D).

The biochemical analysis showed that apocynin treatment reversed the increase in SOD unit in the prefrontal cortex [Fig. 6A, t(8) = 6.043, P < 0.01] and in the striatum [Fig. 6G, t(8) = 10.190, P < 0.0001] but not in the hippocampus [Fig. 6D, t(8) = 2.181, P = 0.06]. Apocynin also reduced the H_2O_2 levels in the prefrontal cortex [Fig. 6B, t(9) = 2.751, P < 0.05], hippocampus [Fig. 6E, t(9) = 4.222, P < 0.01] and in the striatum [Fig. 6H, t(9) = 8.928, P < 0.0001]. However, the CAT activity was further reduced after

the apocynin treatment in the prefrontal cortex [Fig. 6C, t(10) = 4.196, P < 0.01] and hippocampus [Fig. 6F, t(10) = 2.328, P < 0.05], without differences in the striatum [Fig. 4I, t(10) = 1.164, P = 0.27] when compared to CORT+VEH group samples.

Still in the phase 2, we investigated the effect of apocynin alone, without corticosterone administration. No behavioral changes were observed after apocynin treatment in the FST [Fig. 7A, t(14) = 1.741, P = 0.10], TST [Fig. 7B, t(14) = 0.441, P = 0.66] and SPT [Fig. 7C, t(14) = 0.208, P = 0.83] or locomotor changes in the OFT [Fig. 7D]. In the biochemical analyses, no significant changes in SOD enzyme activity were observed in the prefrontal cortex [Fig. 8A, t(8) = 1.820, P = 0.10], hippocampus [Fig. 8D, t(8) = 0.339, P = 0.74] or striatum [Fig 8G, t(8) = 1.758, P = 0.12]. The H_2O_2 levels also did not differ significantly between VEH+VEH and VEH+APO groups in the prefrontal cortex [Fig. 8B, t(8) = 0.526, P = 0.61], hippocampus [Fig. 8E, t(8) = 1.990, P = 0.08] and striatum [Fig. 8H, t(8) = 0.241, P = 0.81]. Only a decrease in CAT enzyme activity was observed in the prefrontal cortex [Fig. 8C, t(10) = 3.378, P < 0.01] and striatum [Fig. 8I, t(10) = 2.246, P < 0.05] without changes in the hippocampus [Fig. 8F, t(10) = 0.246, P = 0.81] of the VEH+APO group.

4. Discussion

Although it is still unclear how oxidative stress is related to depressive symptoms, it is known that the brain is especially vulnerable to oxidative damage [17]. In this work, we evaluated the antidepressant-like effect of apocynin on mice submitted to CCA as well as its effects on brain oxidative profile after this animal protocol of depression. First, we showed the behavioral and the oxidative changes in mice exposed to CCA. Mice of the CORT group showed higher immobility time and lower grooming behavior on the behavioral tests than mice of Vehicle group, characterizing the depressive-like behavior. These mice also presented high SOD activity as well as H_2O_2 levels and CAT

activity reduced in the prefrontal cortex, hippocampus and striatum. Seven-day treatment with apocynin, a plant-derived antioxidant molecule, was able to reverse the most of these changes, suggesting its antidepressant-like effect. In addition, we evaluated the *per se* effect of apocynin on mice not exposed to CCA, but few changes were observed.

The depressive-like behavior accompanied by oxidative stress has already been suggested after other protocols. Camargo et al. (2018) and Zeni et al (2017) demonstrated that after oral Cort administration in mice their brain levels of malondialdehyde (MDA, lipid peroxidation end product), nitrite (example of RNS) and protein carbonyl (relevant marker of protein oxidation) increased [52,53]. Additionally, Lopes et al. (2018) used the same induction protocol as us and, in addition to observing increased MDA and nitrite levels in the prefrontal cortex, hippocampus and striatum, the authors also noted a reduction in reduced glutathione (GSH) levels, an important antioxidant agent. This antioxidant depletion has also been reported after chronic unpredictable stress (CUS) protocol and acute restraint stress (ARS), suggesting that, regardless of the induction protocol used, oxidative stress seems to be present in the depressive-like behavior [54,55].

Even though H_2O_2 is not a free radical, it is an extremely reactive species. It is involved in $HO^{\bullet}$ generation, the most reactive of free radicals. In addition, H_2O_2, unlike free radicals, has a long life and can cross cell membranes, exerting a potentially toxic effect on cells [56]. Afsar et al. (2017) also found high levels of this species in rats brain following CUS protocol. Moreover, the authors observed increased levels of MDA and nitrite and lower antioxidant enzyme levels concurrently with the behavioral changes characteristic of depressive-like behavior [57]. Increased plasma H_2O_2 levels accompanied by reduced activity of antioxidant enzymes in erythrocytes were also

seen in depressed patients [58]. These results reinforce the idea of involving a redox imbalance in the pathophysiology of depression.

Regarding the activity of antioxidant enzymes in brain tissue, while most behavioral studies report reduced CAT activity after induction protocol, findings regarding SOD are divergent [57,59–61]. Yang et al (2018) found reduced levels of rat hippocampal SOD after induction of depressive-like behavior by LPS [62]. Similar results were found in the cerebral cortex and hippocampus of mice subjected to acute restraint stress (ARS) protocol [59]. However, other studies have shown results like as ours, with increased SOD in the mice hippocampus after 4-, 7- and 8-hours restraint sessions [60,61,63]. In addition, increased red blood cell SOD levels have already been reported in patients with major depressive disorder and this parameter was positively correlated with the severity of the disorder [64].

Additionally, NADPH oxidase is proposed as an important source of ROS in various psychiatric disorders [65]. Seo et al. (2012) reported that this enzymatic complex mediates depressive-like behavior induced by chronic restraint stress in mice [66]. Our results also suggest the involvement of NADPH oxidase in depressive-like behavior induced by chronic corticosterone administration. This may be justified by apocynin treatment, which promoted behavioral improvement associated with the restoration of oxidative parameters evaluated. The up-regulation of NADPH oxidase seen in conditions with oxidative stress, such as depression, generates $O_2^{\bullet-}$ excess, substrate of the SOD enzyme [66,67]. Thus, the conversion of this anion to H_2O_2 is stimulated, which remains at high levels due to the impairment of the CAT enzyme activity. This creates an oxidative environment that can be extremely harmful to important mood-related structures such as prefrontal cortex, hippocampal, and striatum. In addition, like apocynin, other antioxidant compounds such as resveratrol

and fish oil have also shown potential antidepressant effects as well as antidepressant drugs such as fluoxetine, imipramine and trazodone have already demonstrated antioxidant properties [68–71].

5. Conclusions

Based on our results, it is suggested that apocynin exerts antidepressant-like effect, inhibiting the NADPH oxidase complex and attenuating the behavioral and oxidative changes induced by CCA. As perspectives it is necessary to intensify the studies so that in the future apocynin and/or other antioxidant compounds can be an alternative for the treatment of this disabling disorder.

Acknowledgements

This work was supported by scholarships from Coordination of Improvement of Higher Education Personnel (CAPES/Brazil) and the National Council for Scientific and Technological Development (CNPq/Brazil).

Declaration of Interest

The authors declare they have no conflict of interest.

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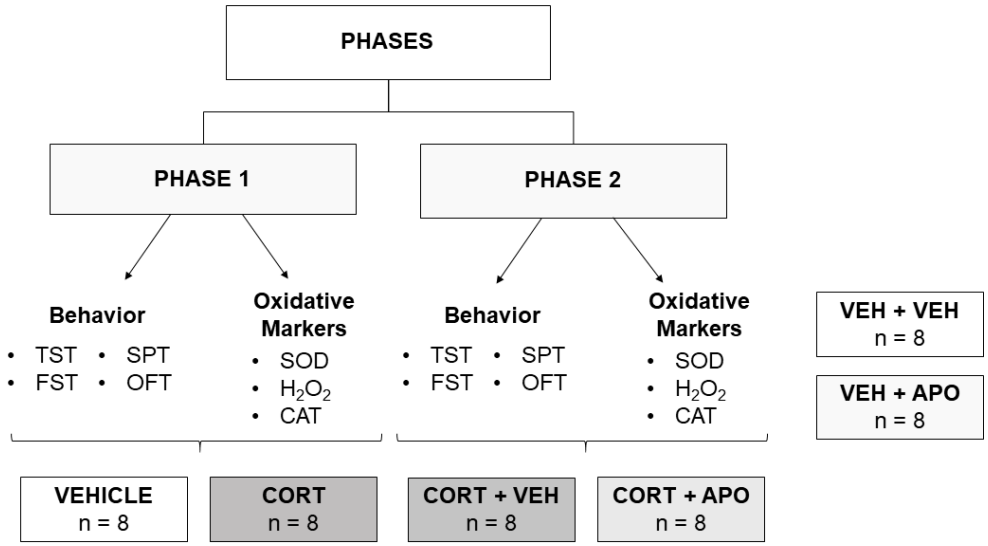


Fig. 1. Experimental design. The study was carried out in two phases: phase 1, which included the induction of depressive-like behavior by the chronic corticosterone administration and assessment of behavioral and oxidative changes after this induction model of depression; and phase 2, which investigated the antidepressant-like effect of apocynin and its effect on oxidative changes in the brain of mice exposed to corticosterone. In addition to this phase, the *per se* effect of apocynin was also evaluated, investigating its effect on the behavior and oxidative parameters in mice not exposed to corticosterone (n= 8/group). Abbreviations: TST: tail suspension test; FST: forced swimming test; SPT: splash test; OFT: open field test; SOD: superoxide dismutase enzyme; H_2O_2: hydrogen peroxide; CAT: catalase enzyme. VEHICLE: mice that received vehicle subcutaneously, once a day, for 21 days; CORT: mice that received corticosterone (20 mg/Kg) subcutaneously, once a day, for 21 days; CORT+VEH: mice that received corticosterone (20 mg/Kg) subcutaneously, once a day, for 21 days and treatment with vehicle orally (gavage) in the last 7 days of the protocol; CORT+APO: mice that received corticosterone (20 mg/Kg) subcutaneously, once a day, for 21 days and treatment with apocynin (100 mg/Kg) orally (gavage) in the last 7 days of the protocol; VEH+VEH: mice that received vehicle subcutaneously, once a day, for 21 days and treatment with vehicle orally (gavage) in the last 7 days of the protocol; VEH+APO: mice that received vehicle subcutaneously, once a day, for 21 days and treatment with apocynin (100 mg/Kg) orally (gavage) in the last 7 days of the protocol.

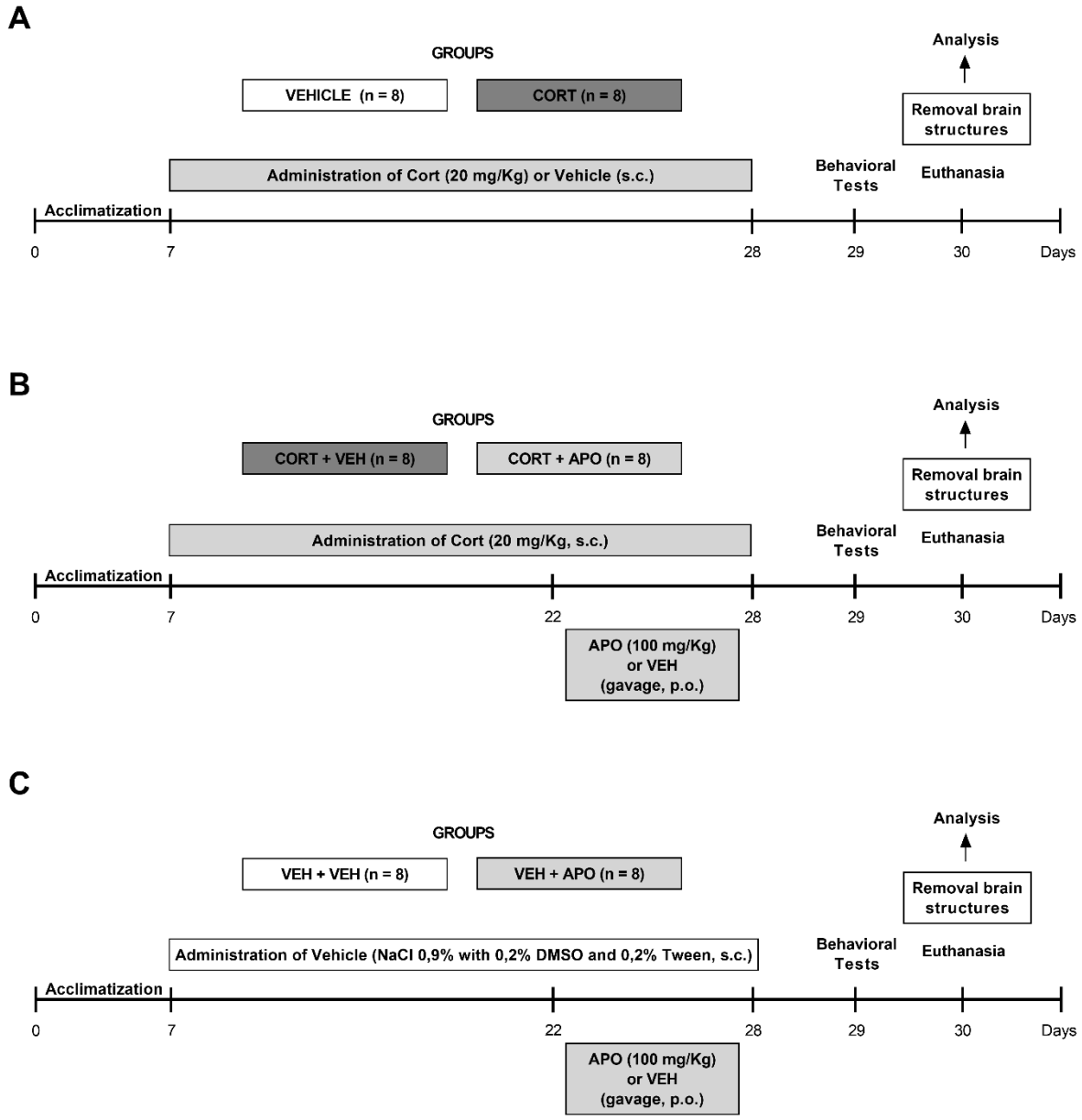
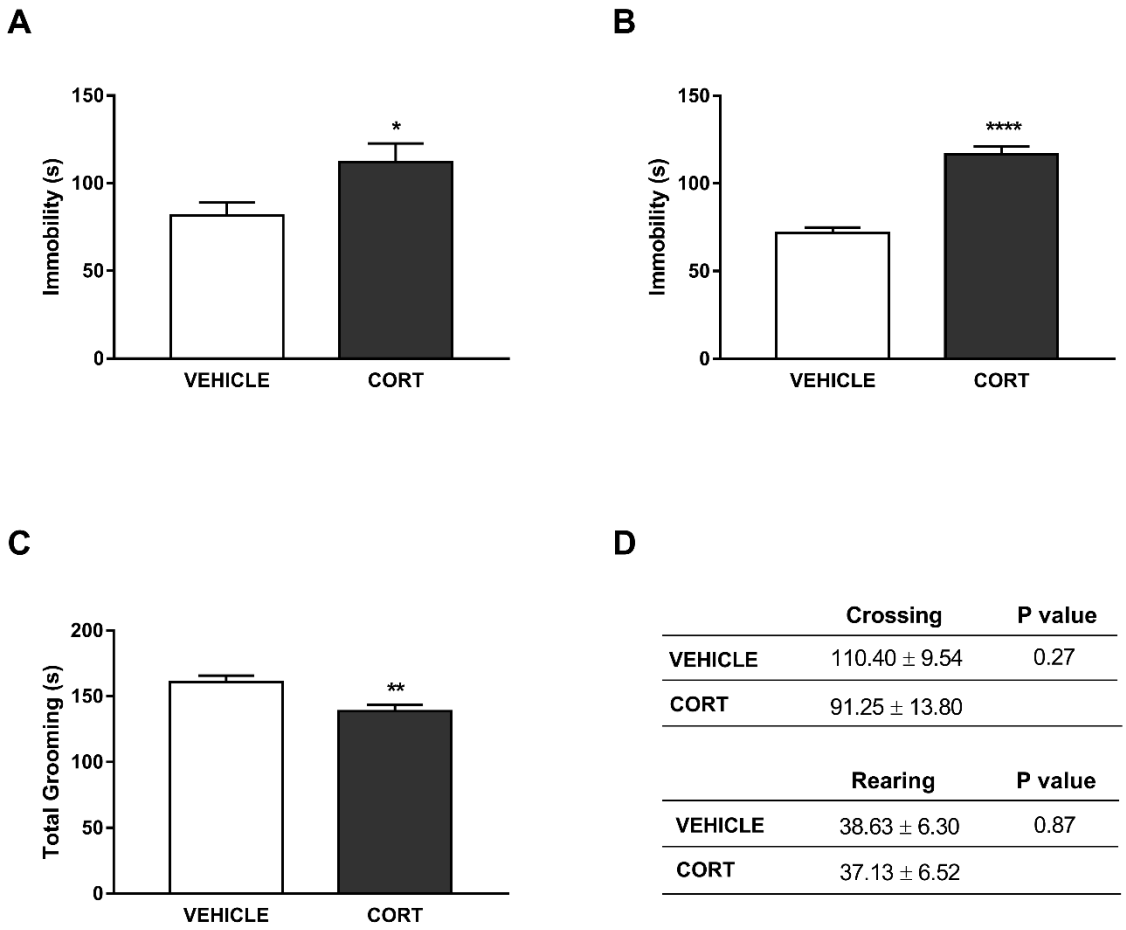


Fig. 2. Experiment timelines. A) Phase 1: Depressive-like behavior induction by the chronic corticosterone administration to investigate behavioral changes in mice and oxidative brain parameters. B) Phase 2: Investigation of the antidepressant-like effect of apocynin and its effect on oxidative brain parameters. C) Complement of Phase 2: Evaluation of the *per se* effect of apocynin in mice not exposed to corticosterone. VEHICLE: mice that received vehicle subcutaneously, once a day, for 21 days; CORT: mice that received corticosterone (20 mg/Kg) subcutaneously, once a day, for 21 days; CORT+VEH: mice that received corticosterone (20 mg/Kg) subcutaneously, once a day, for 21 days and treatment with vehicle orally (gavage) in the last 7 days of the protocol; CORT+APO: mice that received corticosterone (20 mg/Kg) subcutaneously, once a day, for 21 days and treatment with apocynin (100 mg/Kg) orally (gavage) in the last 7 days of the protocol; VEH+VEH: mice that received vehicle subcutaneously, once a day, for 21 days and treatment with vehicle orally (gavage) in the last 7 days of the protocol; VEH+APO: mice that received vehicle subcutaneously, once a day, for 21 days and treatment with apocynin (100 mg/Kg) orally (gavage) in the last 7 days of the protocol.



D

	Crossing	P value
VEHICLE	110.40 ± 9.54	0.27
CORT	91.25 ± 13.80	

	Rearing	P value
VEHICLE	38.63 ± 6.30	0.87
CORT	37.13 ± 6.52	

Fig. 3. Behavioral changes after chronic corticosterone administration. A) Immobility time in the forced swimming test. B) Immobility time in the tail suspension test. C) Time spent on grooming behavior in the splash test. D) Locomotor activity (crossing and rearing) in the open field test. Data are present as the means ± SEM (n=8/group). * $P < 0.05$; ** $P < 0.01$; **** $P < 0.0001$ when compared to vehicle group. The data obtained were analyzed using Student's *t*-test. VEHICLE: group that received vehicle subcutaneously, once a day, for 21 days; CORT: mice that received corticosterone (20 mg/Kg) subcutaneously, once a day, for 21 days.

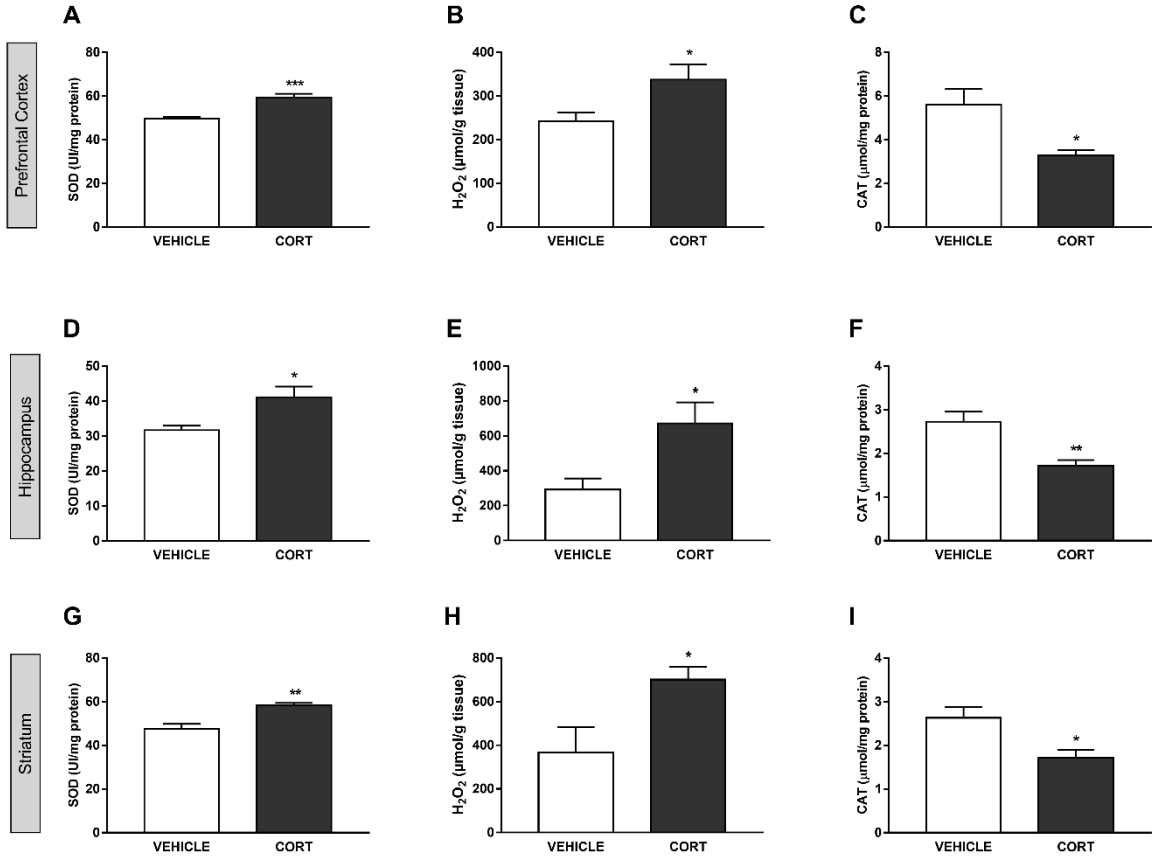
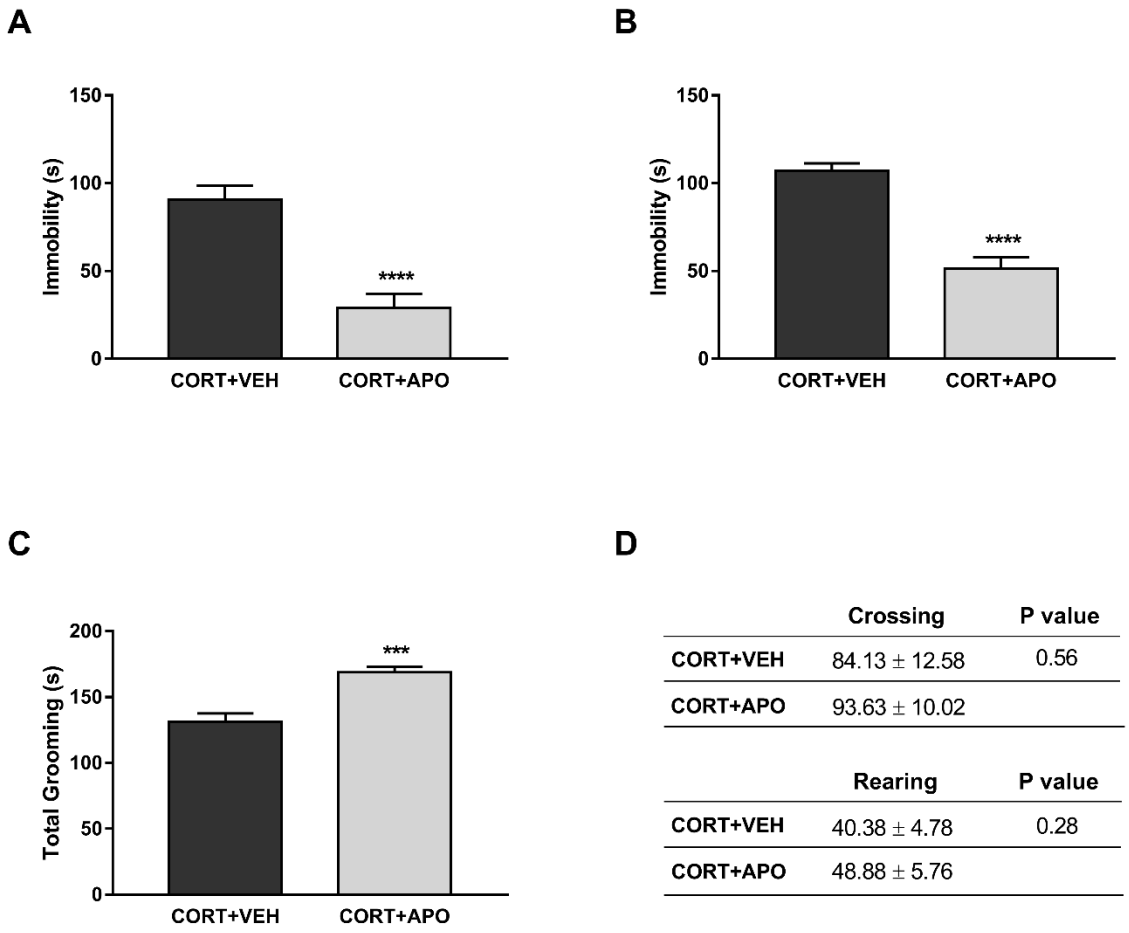


Fig. 4. Brain oxidative profile after chronic corticosterone administration. A) Superoxide dismutase (SOD)-like activity B) hydrogen peroxide (H_2O_2) levels and C) catalase (CAT) activity in the prefrontal cortex. D) SOD-like activity E) H_2O_2 levels and F) CAT activity in the hippocampus. G) SOD-like activity and H) H_2O_2 levels in the striatum. Data are present as the means ± SEM (n= 4–7/group). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ when compared to vehicle group. The data obtained were analyzed using Student's *t*-test. VEHICLE: group that received vehicle subcutaneously, once a day, for 21 days; CORT: mice that received corticosterone (20 mg/Kg) subcutaneously, once a day, for 21 days.



	Crossing	P value
CORT+VEH	84.13 ± 12.58	0.56
CORT+APO	93.63 ± 10.02	

	Rearing	P value
CORT+VEH	40.38 ± 4.78	0.28
CORT+APO	48.88 ± 5.76	

Fig. 5. Antidepressant-like effect of apocynin. A) Immobility time in the forced swimming test. B) Immobility time in the tail suspension test. C) Time spent on grooming behavior in the splash test. D) Locomotor activity (crossing and rearing) in the open field test. Data are present as the means ± SEM (n=8/group). *** P < 0.001; **** P < 0.0001 when compared to CORT+VEH group. The data obtained were analyzed using Student's *t*-test. CORT+VEH: group that received corticosterone (20 mg/Kg) subcutaneously, once a day, for 21 days and treatment with vehicle orally (gavage) in the last 7 days of protocol; CORT+APO: group that received corticosterone (20 mg/Kg) subcutaneously, once a day, for 21 days and treatment with apocynin (100 mg/Kg) orally (gavage) in the last 7 days of the protocol.

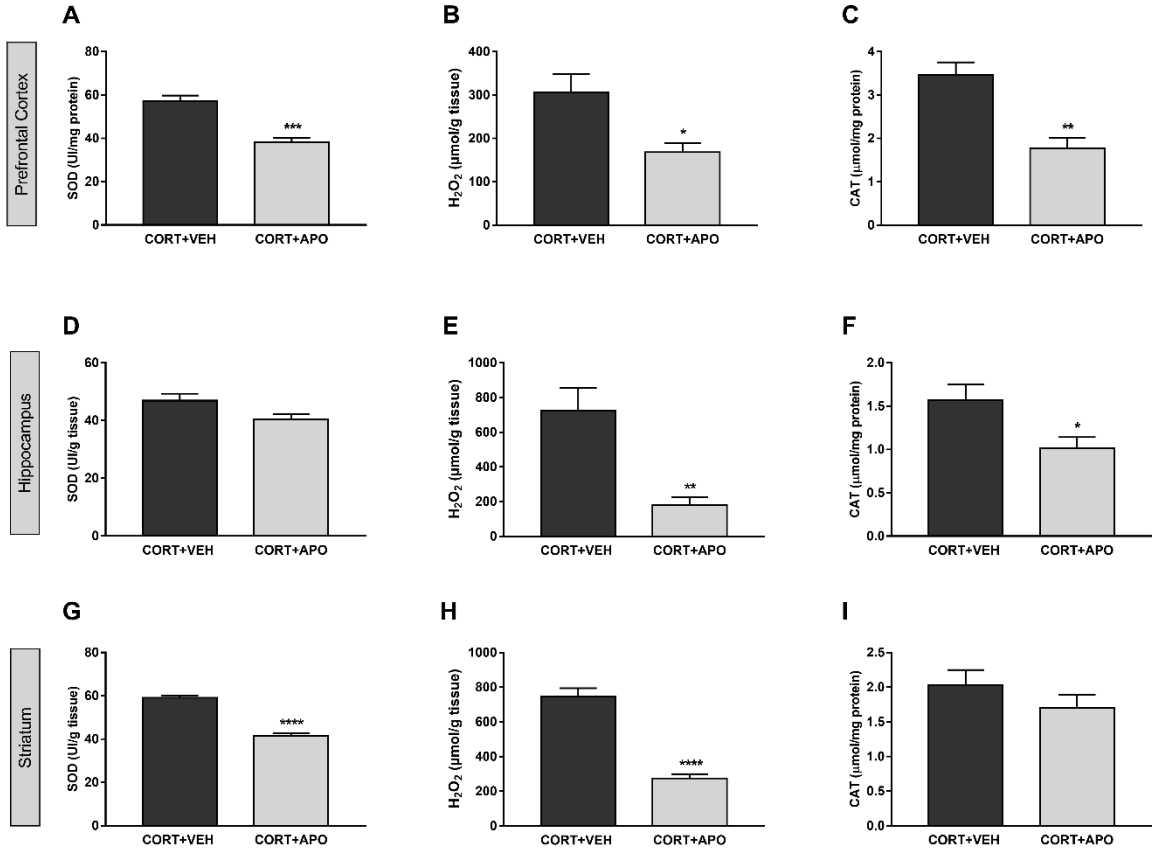
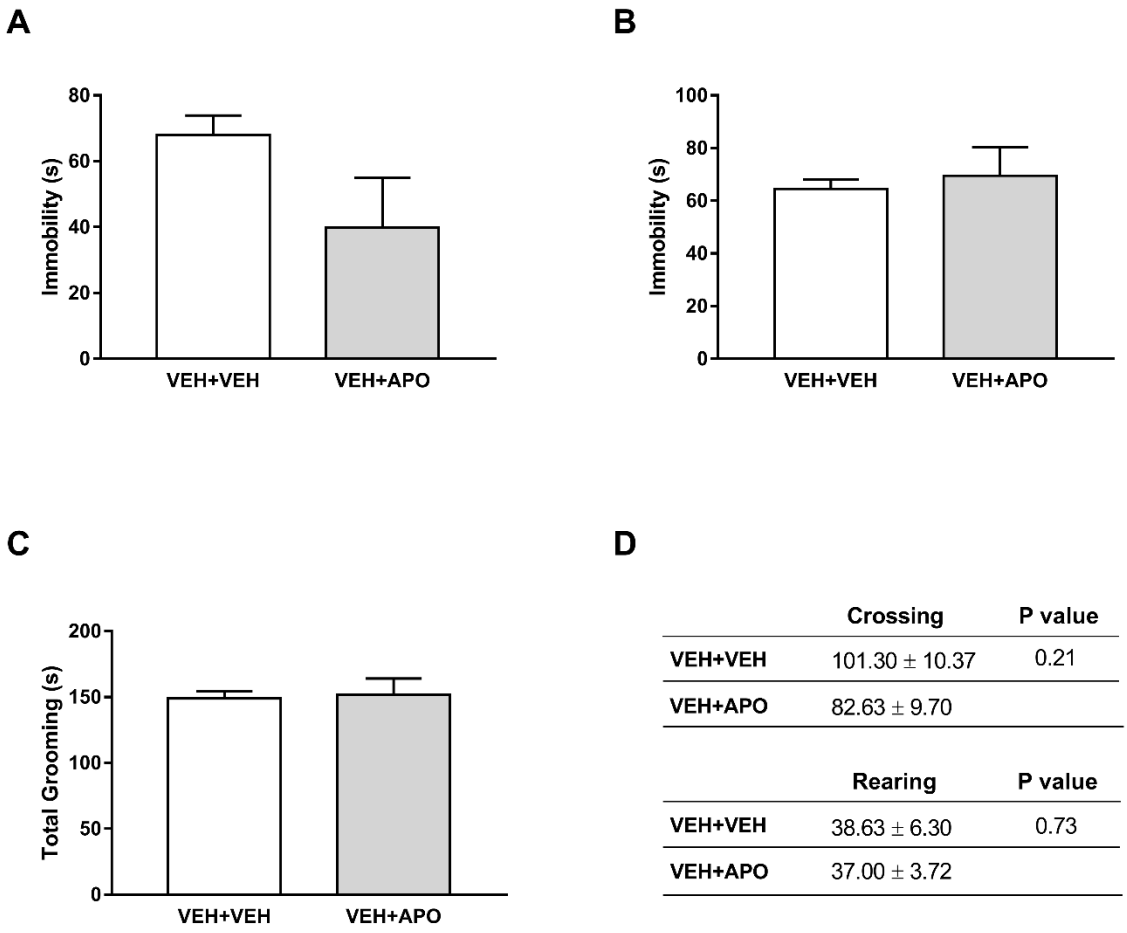


Fig. 6. Effect of apocynin on oxidative changes in the brain of mice exposed to corticosterone. A) Superoxide dismutase (SOD)-like activity B) hydrogen peroxide (H_2O_2) levels and C) catalase (CAT) activity in the prefrontal cortex. D) SOD-like activity E) H_2O_2 levels and F) CAT activity in the hippocampus. G) SOD-like activity and H) H_2O_2 levels in the striatum. Data are present as the means ± SEM (n= 4–7/group). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$ when compared to CORT+VEH group. The data obtained were analyzed using Student's *t*-test. CORT+VEH: group that received corticosterone (20 mg/Kg) subcutaneously, once a day, for 21 days and treatment with vehicle orally (gavage) in the last 7 days of protocol; CORT+APO: group that received corticosterone (20 mg/Kg) subcutaneously, once a day, for 21 days and treatment with apocynin (100 mg/Kg) orally (gavage) in the last 7 days of the protocol.



D

	Crossing	P value
VEH+VEH	101.30 ± 10.37	0.21
VEH+APO	82.63 ± 9.70	

	Rearing	P value
VEH+VEH	38.63 ± 6.30	0.73
VEH+APO	37.00 ± 3.72	

Fig. 7. Effect of apocynin on the behavior of mice not exposed to corticosterone. A) Immobility time in the forced swimming test. B) Immobility time in the tail suspension test. C) Time spent on grooming behavior in the splash test. D) Locomotor activity (crossing and rearing) in the open field test. Data are present as the means ± SEM (n=8/group) and were analyzed using Student's *t*-test. VEH+VEH: mice that received vehicle subcutaneously, once a day, for 21 days and treatment with vehicle orally (gavage) in the last 7 days of the protocol; VEH+APO: mice that received vehicle subcutaneously, once a day, for 21 days and treatment with apocynin (100 mg/Kg) orally (gavage) in the last 7 days of the protocol.

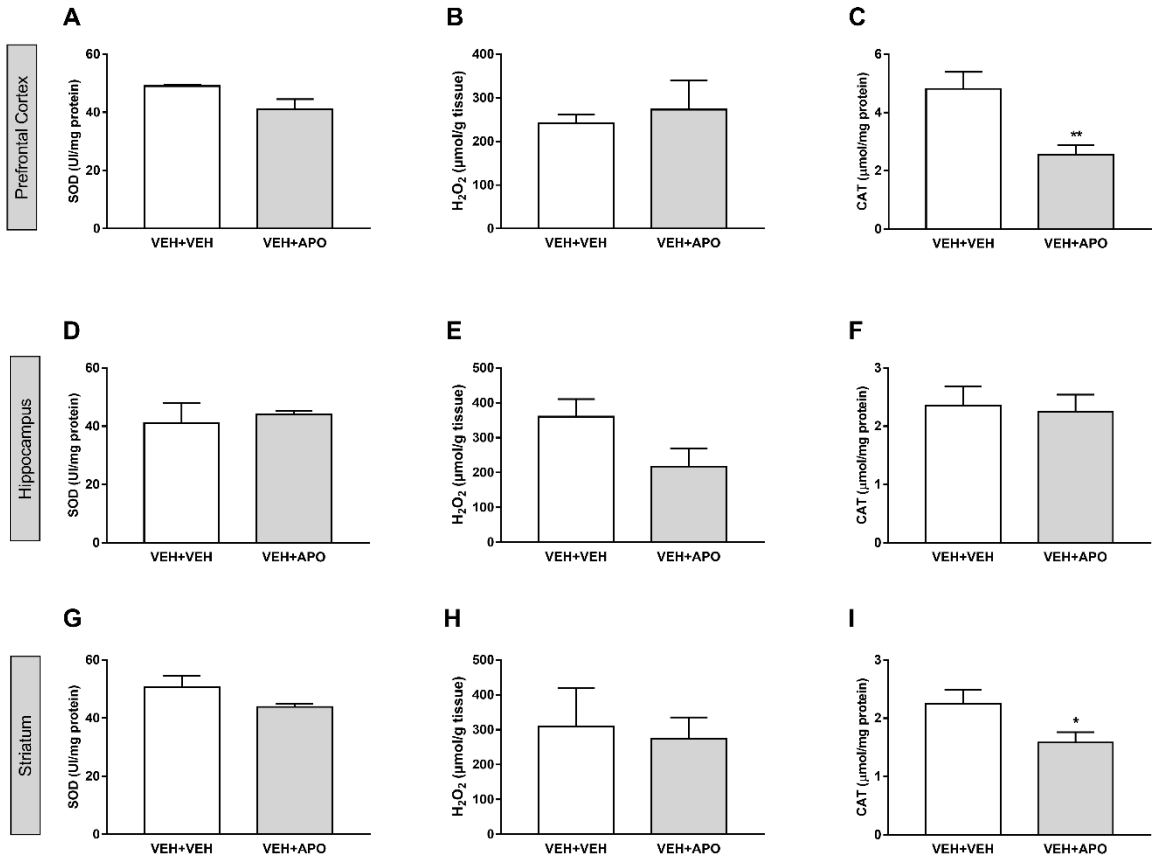
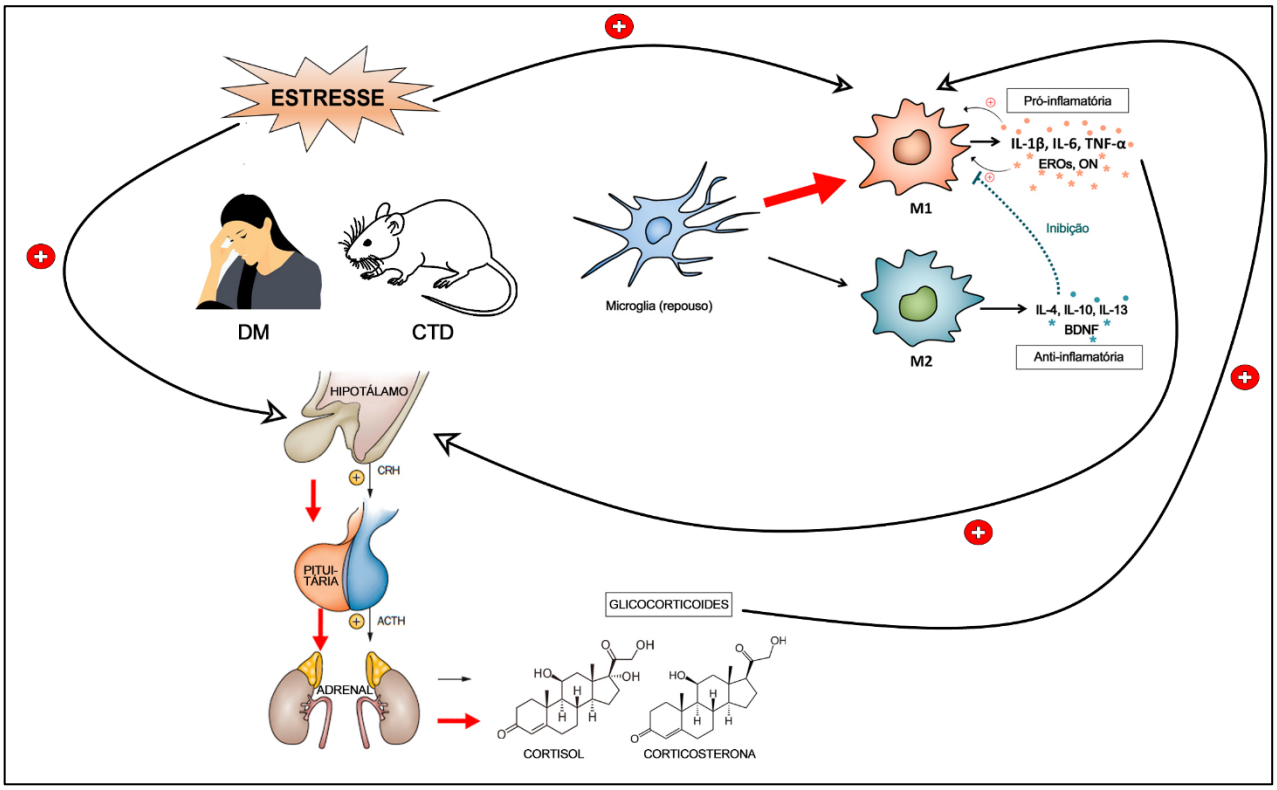


Fig. 8. Effect of apocynin on the brain oxidative parameters of mice not exposed to corticosterone. A) Superoxide dismutase (SOD)-like activity B) hydrogen peroxide (H_2O_2) levels and C) catalase (CAT) activity in the prefrontal cortex. D) SOD-like activity E) H_2O_2 levels and F) CAT activity in the hippocampus. G) SOD-like activity and H) H_2O_2 levels in the striatum. Data are present as the means ± SEM (n= 4–7/group). * $P < 0.05$; ** $P < 0.01$ when compared to VEH+VEH group. The data obtained were analyzed using Student's *t*-test. VEH+VEH: mice that received vehicle subcutaneously, once a day, for 21 days and treatment with vehicle orally (gavage) in the last 7 days of the protocol; VEH+APO: mice that received vehicle subcutaneously, once a day, for 21 days and treatment with apocynin (100 mg/Kg) orally (gavage) in the last 7 days of the protocol.

4. DISCUSSÃO GERAL

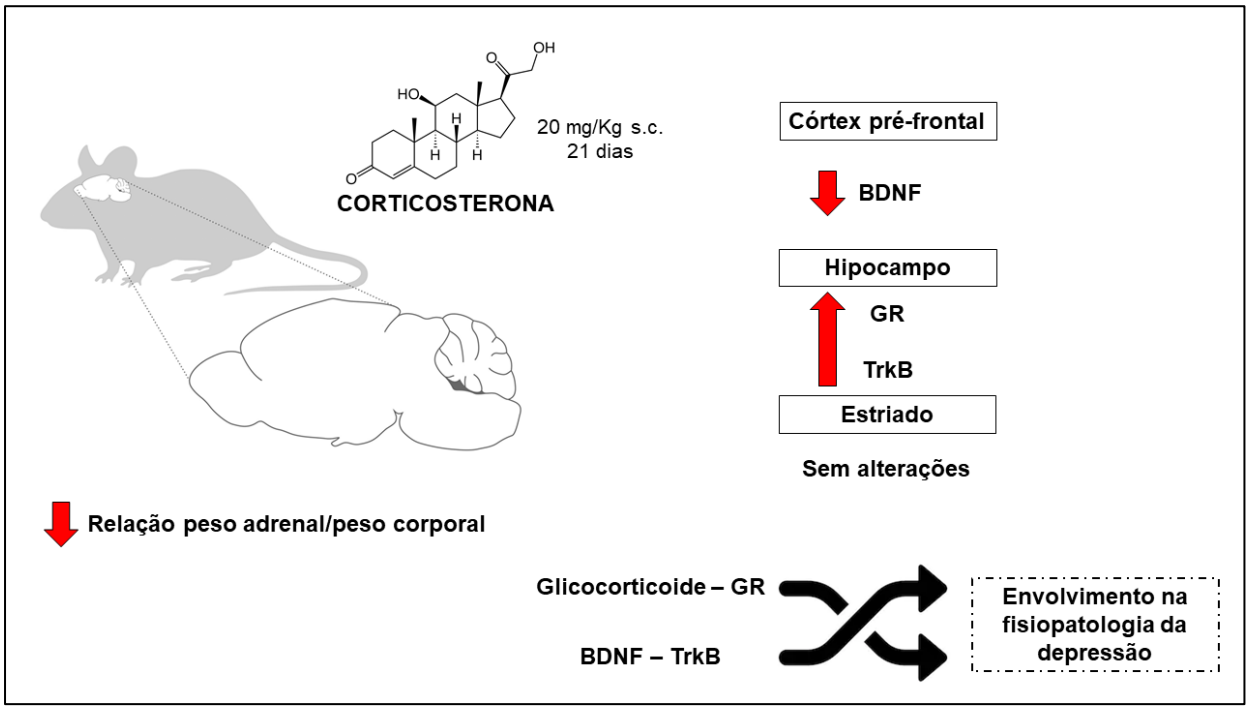
Além de apresentar origem multifatorial, a depressão parece envolver uma gama de mecanismos fisiopatológicos. No manuscrito 1 (item 3.1), o compilado de dados apresentados destaca a participação e relação do sistema imune, através das células microgliais, e do principal sistema de resposta ao estresse, o eixo HPA (Figura 1). No manuscrito 2 (item 3.2), os resultados demonstram experimentalmente a desregulação do eixo HPA, assim como a redução dos níveis de BDNF, sugerindo prejuízo na neurogênese de camundongos com CTD induzido por corticosterona (Figura 2). Por fim, o manuscrito 3 (item 3.3) descreve o perfil oxidativo após o modelo de ACC e uma possível alternativa de tratamento com apocinina, um antioxidante natural (Figura 3).

Figura 1 – Envolvimento e Relação da microglia e eixo HPA na fisiopatologia da depressão.



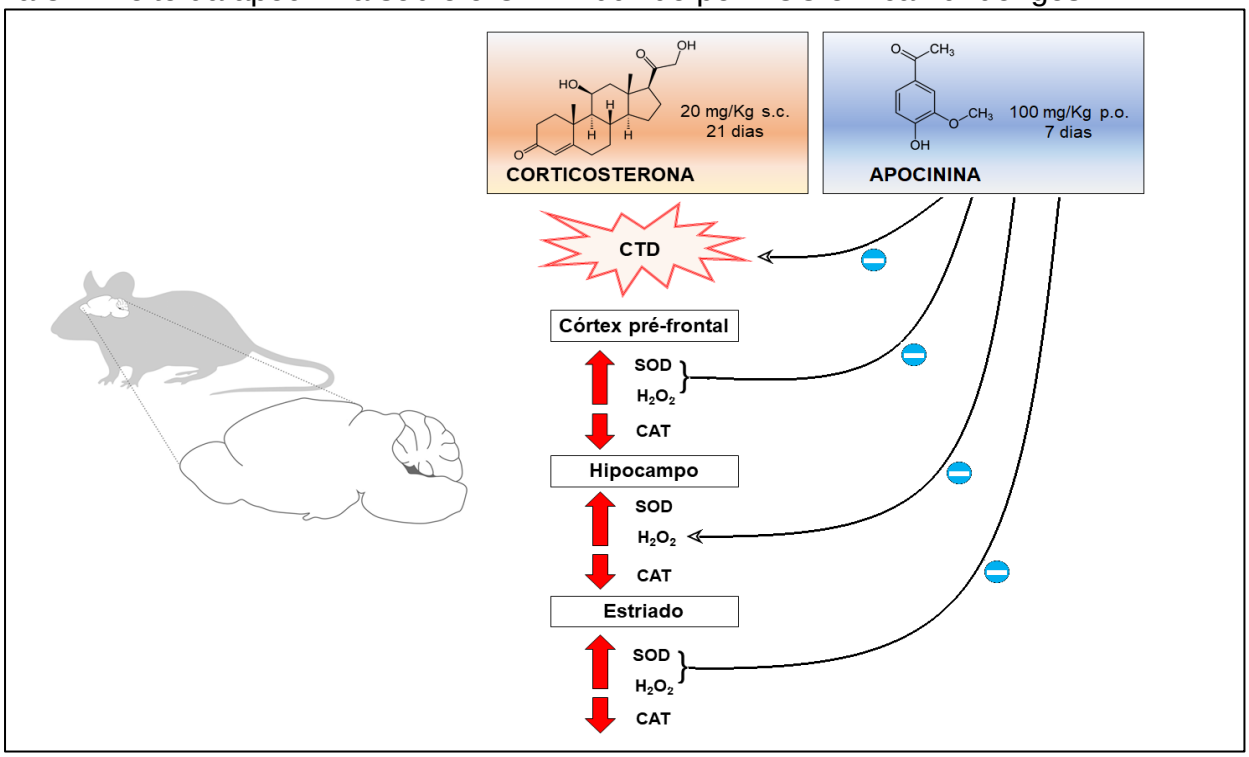
Fonte: Autora.

Figura 2 – Participação e relação das vias de sinalização GC/GR e BDNF/TrkB na fisiopatologia da depressão.



Fonte: Autora.

Figura 3 – Efeito da apocinina sobre o CTD induzido por ACC em camundongos.



Fonte: Autora.

Apesar de apresentados separadamente, nós acreditamos que os mecanismos discutidos no presente trabalho possam ocorrer de maneira simultânea. Como mencionado anteriormente, o estresse consiste em um fator comum aos mecanismos fisiopatológicos da depressão (ANISMAN e MATHESON, 2005). De fato, o estresse estimula tanto a ativação do eixo HPA quanto das células microgliais (THURGUR e PINTEAUX, 2019; TIMMERMANS; SOUFFRIAU e LIBERT, 2019). Como consequência à ativação do eixo neuroendócrino, ocorre uma maior liberação de GCs, podendo gerar hipercortisolismo, condição considerada um fator de risco para o desenvolvimento de depressão (GIBBONS e MCUGH, 1962; COSGRIFF et al., 1990). Além do mais, de 20 a 80% dos pacientes depressivos exibem alguma forma de hiperativação do eixo HPA, apresentando níveis aumentados de ACTH no sangue, assim como de cortisol no sangue, saliva e urina (THASE, JINDAL e HOWLAND, 2002; STETLER e MILLER, 2011; MURRI et al., 2014). Entretanto, no presente trabalho observamos experimentalmente uma redução nos níveis séricos de corticosterona em camundongos submetidos ao protocolo de ACC. Uma explicação sugerida para tal achado é a interferência do glicocorticoide exógeno administrado, o qual pode ter suprimido a produção endógena. Contudo, a desregulação do eixo HPA ainda pode ser sugerida, baseada na observação da redução da relação peso adrenal/peso corporal encontrada após esse protocolo experimental.

Essa desregulação do eixo HPA desencadeada pelo estresse favorece alterações na expressão proteica dos receptores glicocorticoides (GR) e mineralocorticoides (MR). Isso é particularmente relevante em estruturas como o hipocampo e o hipotálamo, as quais regulam esse eixo através de feedback negativo (JURUENA; CLEARE e PARIANTE, 2004; HERMAN et al., 2016). Nossos resultados apontaram um aumento na expressão proteica de GR no hipocampo e isso também pode ser considerado um marcador positivo da ação da corticosterona, a qual a longo prazo pode exercer efeitos neurotóxicos. Dentre esses efeitos estão o prejuízo comportamental, na neurogênese e na neuroplasticidade, os quais estão intimamente relacionados ao desenvolvimento da depressão (KIM e DIAMOND, 2002; ANACKER et al., 2013; JEANNETEAU e CHAO, 2013; KUREK et al., 2016).

A hipersecreção persistente de GCs após a ativação do eixo HPA em algumas situações de estresse também pode sensibilizar as células microgliais, ativando-as (ZHANG et al., 2018). Essa ativação, a qual é predominantemente do subtipo M1, desencadeia um aumento na geração de mediadores inflamatórios e oxidativos

(THURGUR e PINTEAUX, 2019). Esses mediadores também podem influenciar negativamente a neurogênese e a neuroplasticidade, as quais são ainda mais prejudicadas pela redução do BDNF, observada após condições estressantes e na DM (DOWLATI, 2010; AUTRY e MONTEGGIA, 2012). Corroborando com isso, observamos expressão proteica reduzida de BDNF após o protocolo experimental de ACC no córtex pré-frontal, outra estrutura cerebral relacionada aos processos cognitivos e emocionais.

Adicionalmente à estimulação das células microgliais pelos glicocorticoides, as citocinas pró-inflamatórias e espécies reativas liberadas após a ativação microglial podem, da mesma forma, ativar o eixo HPA, formando um ciclo bidirecional (COLTON, 2009). Nesse contexto, moduladores da atividade microglial e dos receptores GR e MR, assim como alguns agentes antioxidantes como a apocinina, têm se mostrado promissores para o tratamento de depressão (ANACKER et al, 2011; NABAVI et al., 2018; ZHANG et al., 2019). No presente trabalho, o manuscrito 3 descreve experimentalmente o efeito tipo-antidepressivo da apocinina após o protocolo de ACC, sugerindo como mecanismo de ação a modulação de parâmetros oxidativos alterados pela ação crônica da corticosterona. Portanto, o conjunto de dados discutidos acima sugere a integração de diferentes vias na depressão, evidenciando não só a sua complexidade mas também incentivando a busca por novos esclarecimentos, assim como por moduladores para tratar essa condição incapacitante.

5. CONCLUSÃO

- A depressão pode ser caracterizada como um distúrbio psiquiátrico “multifisiopatológico”;
- Tanto o estresse oxidativo, quanto os sistemas neurotrófico (representado pelo BDNF), imunológico e neuroendócrino estão envolvidos na fisiopatologia da depressão;
- Todos os processos fisiopatológicos discutidos parecem estar interligados;
- A apocinina constitui uma boa candidata para o tratamento desse transtorno.

Perspectivas futuras: dar continuidade ao estudo das vias alteradas assim como testar possíveis moduladores das mesmas, visando melhorar o tratamento desse transtorno e, consequentemente, a qualidade de vida dos pacientes depressivos.

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ANEXO A: Carta de aprovação da Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria.

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

CERTIFICADO

Certificamos que a proposta intitulada "ENVOLVIMENTO DO CANAL TRPA1 NA FISIOPATOLOGIA DA DEPRESSÃO EM CAMUNDONGOS", protocolada sob o CEUA nº 7698200617, sob a responsabilidade de **Guilherme Vargas Bochi** *e equipe; Gabriela Trevisan dos Santos; Patricia Severo do Nascimento; Rafael Noal Moresco* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (CEUA/UFSM) na reunião de 25/08/2017.

We certify that the proposal "TRPA1 CHANNEL INVOLVEMENT IN PATHOPHYSIOLOGY OF DEPRESSION IN MICE", utilizing 192 Heterogenics mice (192 males), protocol number CEUA 7698200617, under the responsibility of **Guilherme Vargas Bochi** *and team; Gabriela Trevisan dos Santos; Patricia Severo do Nascimento; Rafael Noal Moresco* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM) in the meeting of 08/25/2017.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 08/2017 a 04/2019 Área: Farmacologia

Origem:	Biotério Central UFSM						
Espécie:	Camundongos heterogênicos	sexo:	Machos	idade:	50 a 60 dias	N:	192
Linhagem:	Swiss			Peso:	20 a 30 g		

Resumo: Segundo a Organização Mundial da Saúde, mais de 300 milhões de pessoas sofrem de depressão no mundo. Esses casos correspondem a aproximadamente 4,4 % da população mundial. Considerando que a depressão é a segunda maior responsável pelas causas de morte por suicídio e que grande parcela de pacientes depressivos são refratários a qualquer um dos agentes antidepressivos (aproximadamente 30%), é de grande importância o entendimento dos mecanismos envolvidos na fisiopatologia da depressão. O receptor de potencial transitório anquirina 1 (TRPA1) faz parte de uma ampla família de canais iônicos não seletivos (TRP) e age como um sensor para mediadores inflamatórios e compostos oxidantes. Tem sido sugerido que mediadores inflamatórios e oxidativos contribuem para a neuroinflamação e suprimem a neurogênese no sistema nervoso central (SNC), estando envolvidos na fisiopatologia da doença. No entanto, apesar dos avanços sobre a fisiopatogênese da depressão, nenhum mecanismo estabelecido pode explicar todos os aspectos da doença. Assim, o objetivo deste projeto é investigar o envolvimento do canal TRPA1 no desenvolvimento de comportamento-semelhante a depressão em modelos de depressão em camundongos. Para isso, será utilizado o modelo de Estresse induzido pela Administração de Corticosterona (AC) para induzir comportamentos semelhantes a depressão nos animais. Através deste modelo, serão avaliados os efeitos de antagonistas seletivos do TRPA1 (HC-030031 e A-967079) sobre parâmetros comportamentais de depressão, bem como sobre mediadores inflamatórios e oxidativos envolvidos na desordem depressiva.

Local do experimento: Laboratório de Pesquisa Experimental da Fisiopatologia da Dor.

Santa Maria, 07 de fevereiro de 2018

Prof. Dr. Denis Broock Rosemberg
Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Prof. Dr. Saulo Tadeu Lemos Pinto Filho
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Avenida Roraima, 1000, Reitoria, 2º andar - CEP 97105-900 Santa Maria, RS - tel: 55 (55) 3220-9362 / fax:
Horário de atendimento: das 8:30 ás 12h e 14h ás 17hs : e-mail: ceua.ufsm@gmail.com
CEUA N 7698200617

ANEXO B: Comprovante de aceite do manuscrito 2

Date: Dec 30, 2019
To: "Guilherme Bochi" guilherme.bochi@ufsm.br
From: "Behavioural Pharmacology" bpharm@lww.co.uk
Subject: Behavioural Pharmacology Decision

Dec 30, 2019

RE: BP-19-166R1, entitled "Glucocorticoid and BDNF relationship: a brief investigation into the model of depression by chronic administration of corticosterone"

Dear Dr Bochi,

I am pleased to inform you that your paper has now been accepted for publication in Behavioural Pharmacology. All manuscript materials will be forwarded to the production staff for placement in the next available issue and for on-line early publication.

There is an Open Access option for authors. This not mandatory: it is your choice. Please see below for details.

Thank you for submitting your interesting and important work to this journal.

With kind regards,

Dr Jack Bergman
Editor
Behavioural Pharmacology

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b. Manuscript Number - BP-19-166R1

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