PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
FACULDADE DE BIOCIÊNCIAS
PÓS-GRADUAÇÃO EM BIOLOGIA CELULAR E MOLECULAR
DOUTORADO EM BIOLOGIA CELULAR E MOLECULAR

Efeitos neuroimunoendócrinos do estresse por abuso físico ou negligência na infância em mulheres com depressão maior

Tese apresentada ao Programa de Pós-Graduação em Biologia Celular e Molecular da Pontifícia Universidade Católica do Rio Grande do Sul como requisito parcial para a obtenção do título de Doutor em Biologia Celular e Molecular.

Rodrigo Pestana Lopes

Prof. Dr. Moisés Evandro Bauer
Orientador

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Aprovada em _____ de _____ de _____ .

BANCA EXAMINADORA:

Prof. Dr. Diogo Rizzato Lara – PUCRS

Prof. Dr. Aldo Bolten Lucion – UFRGS

Prof. Dr. José Artur Bogo Chies - UFRGS

Dedico esta tese à minha família, que tanto me apoiou, incentivou e contribuiu para que meu crescimento profissional fosse a materialização de um desejo consciente.

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RESUMO

INTRODUÇÃO: Sabe-se que experiências traumáticas na infância podem levar ao surgimento de distúrbios psiquiátricos na vida adulta, incluindo a depressão maior (DM). Contudo, ainda se desconhece o grau de alterações biológicas que eventos estressores traumáticos vivenciados na infância podem produzir em indivíduos com depressão. OBJETIVOS: Investigar parâmetros neuroendócrinos e imunológicos em mulheres adultas deprimidas, com sintomas de Estresse Pós-Traumático (TEPT) e história de abuso e negligência infantil (ANI). MÉTODOS: Trinta e oito mulheres com DM com ou sem história de abuso e negligência infantil e sintomas de TEPT e 19 mulheres saudáveis fizeram parte da coleta de dados desta tese. Os resultados deste trabalho foram divididos em 3 artigos científicos originais e uma revisão do tema. As avaliações incluíram dosagens de níveis salivares de cortisol e de sulfato de dehidroepiandrosterona (DHEAS) por radioimunoensaio; a mitogênese induzida de linfócitos T de sangue periférico foi avaliada por ensaio colorimétrico, bem como a sensibilidade de linfócitos T a moduladores sintéticos (dexametasona) e naturais (epinefrina e sulfato de dehidroepiandrosterona); a secreção de citocinas de perfis Th1/Th2 (IL-2, IL-4, IL-6, IL-10, TNF-a, IFN-g) por células mononucleares foi identificada por citometria de fluxo; e os níveis plasmáticos de BDNF, além de TNF-a e seus receptores solúveis (sTNFR1 e sTNFR2), foram identificados por ELISA. RESULTADOS: Pacientes deprimidas com ou sem trauma infantil apresentaram níveis reduzidos e semelhantes de cortisol salivar e DHEAS em paralelo com proliferação reduzida de linfócitos T. As células mononucleares de sangue periférico das pacientes deprimidas foram menos sensíveis à dexametasona (DEX) ou epinefrina (EPI) e produziram níveis significativamente reduzidos de IL-2, IL-4 e TNF-a quando comparadas ao grupo de controles. As pacientes deprimidas apresentaram ainda níveis plasmáticos elevados de sTNFR1 e sTNFR2, além de redução dos

níveis de BDNF. CONCLUSÕES: Embora muitas alterações biológicas tenham sido identificadas nas mulheres com DM em relação ao grupo de mulheres saudáveis, poucas foram correlacionadas com história de abuso e negligência na infância. Sendo assim, de uma forma geral, conclui-se que a história de abuso e negligência na infância não impacta significativamente as alterações neuroendócrinas e imunológicas apresentadas por pacientes com depressão maior.

ABSTRACT

INTRODUCTION: Traumatic events experienced in childhood may lead to psychiatric diseases in adult life, including major depressive disorder (MDD). It is still obscure to what extent early life stress is associated with biological relevant changes in MDD. OBJECTIVES: To investigate both neuroendocrine and immunological correlates in recurrent MDD with history of childhood maltreatment (CMT) and current PTSD symptoms. METHODS: Thirty-eight female MDD patients with or without childhood trauma and 19 healthy controls took part in this study. Results from this work were presented in 3 original scientific articles and one scientific review. Evaluations included the detection of salivary levels of cortisol and dehydroepiandrosterone sulphate (DHEAS), assessed by radioimmunoassay; induced mitogenesis of isolated T-cells and cellular sensitivity to synthetic (dexametasone) and natural (epinephrine and DHEAS) substances were evaluated by colorimetric assays; Th1/Th2 cytokines (IL-2, IL-4, IL-6, IL-10, TNF-a, IFN-g) were assessed by flow cytometry; plasma levels of BDNF, TNF-a and its soluble receptors (sTNFR1 and sTNFR2) were assessed by ELISA. RESULTS: MDD patients with or without previous trauma had similarly lower salivary cortisol and DHEAS in parallel with blunted T-cell proliferation. PBMCs of depressives were significantly less sensitive to dexamethasone (DEX) or epinephrine (EPI) than controls. PBMCs of MDD patients produced significantly lower IL-2, IL-4 and TNF-α levels when compared to healthy controls. MDD patients also presented higher plasma sTNFR1 and sTNFR2 levels and reduced plasma BDNF. CONCLUSION: Even though several biological changes have been detected in MDD subjects when compared to controls, few alterations were correlated to early life stress. Therefore, we conclude that a history of early life stress did not modify the blunted neuroendocrine and immunological alterations presented by recurrent depressed patients.

SUMÁRIO

1. INTRODUÇÃO

Tanto o cérebro quanto o sistema imune constituem alguns dos sistemas mais adaptáveis do organismo (Sternberg, 2006) e que se comunicam com o objetivo de regular a homeostasia (Tracey, 2002). Os sistemas-chave envolvidos nessa comunicação são o eixo Hipotálamo-Pituitária-Adrenal (HPA) e o Sistema Nervoso Autônomo (SNA), sendo este constituído pelo Sistema Nervoso Simpático (SNS) adrenérgico, pelo Sistema Nervoso Parassimpático (SNP) vago-mediado e pelo Sistema Nervoso Entérico (SNE) (Elenkov et al., 2000, Sternberg, 2006, Tracey, 2002). Durante as últimas décadas, estudos destacaram o eixo HPA e o SNS como as duas principais vias de comunicação entre o cérebro e o sistema imune, por meio da liberação de substâncias (principalmente cortisol e catecolaminas) que regulam a magnitude de respostas imunes inatas e adaptativas (Brogden et al., 2005, Eskandari and Sternberg, 2002, Kohm and Sanders, 2000, Steinman, 2004, Straub, 2004).

A atividade do eixo HPA é ditada pela secreção do hormônio liberador de corticotropina (*corticotropin-releasing hormone* - CRH) pelo hipotálamo. O CRH induz a secreção do hormônio adrenocorticotrópico (*adrenocorticotropic hormone* – ACTH) pela pituitária. O ACTH, por sua vez, estimula a secreção de hormônios corticoesteórides, como os glicocorticóides (cortisol, em humanos), pelas glândulas adrenais (córtex adrenal). Os glicocorticóides (GCs) interagem com seus receptores (*glucocorticoid receptors* - GCRs) em quase todos os tecidos do corpo e em diferentes tipos celulares. Pela ligação a GCRs no cérebro, os GCs também modulam a secreção de CRH pelo hipotálamo e de ACTH pela pituitária, controlando, conseqüentemente, a sua própria concentração na circulação (*feedback* negativo) (Webster et al., 2002).

Mesmo havendo relatos de que todos os hormônios do eixo HPA têm efeito direto sobre a atividade imune celular (Webster et al., 2002), o cortisol é tido como o mais importante deles. Conforme já mencionado, os mecanismos de ação dos GCs se baseiam na ligação desta molécula a GCRs. O cortisol, embora seja um hormônio GC natural que, conseqüentemente, liga-se a GCRs, possui a propriedade de ligar-se também a outro tipo de receptor, o receptor para hormônios mineralocorticóides (*mineralocorticoid receptors* – MCR) (De Kloet et al., 1975). No organismo, os GCs são reguladores essenciais do desenvolvimento, do metabolismo, da homeostasia e de funções efetoras do sistema imune inato e adaptativo (Webster et al., 2002, Sternberg, 2001, Pitzalis et al., 2002, Cancedda et al., 2002). Eles alteram a ativação, diferenciação e maturação de muitos tipos de células imunes, bem como exercem múltiplos papéis na regulação da sensibilidade imune celular à apoptose (Ashwell et al., 2000, Refojo et al., 2001). Além disso, os GCs apresentam também propriedades antiinflamatórias, podendo reduzir efetivamente parâmetros de inflamação como a velocidade de sedimentação globular (VSG) e níveis de proteína C reativa (CRP) (Chikanza et al., 2003). Os GCs sintéticos são potentes drogas imunossupressoras e antiinflamatórias e representam alguns dos medicamentos mais importantes utilizados na terapêutica nos últimos anos (Wan and Nordeen, 2002, Schacke et al., 2002).

A magnitude dos efeitos biológicos dos GCs é determinada, entre outros fatores, pela quantidade de receptores nas células-alvo e pela afinidade dos receptores aos GCs (Schlaghecke et al., 1994). Os efeitos dos GCs se devem principalmente à inibição da liberação de citocinas por células imunes. Após sua ligação aos receptores seletivos, os GCs induzem a transcrição do inibidor de proteínas IκB (IκBα) que mantém o fator nuclear-κB (NFκB) no citoplasma em sua forma inativa, impedindo o NFκB de migrar para o núcleo, onde se ligaria ao elemento de resposta apropriado no DNA e ativaria a produção/secreção de citocinas e contribuiria, dessa

forma, para a imunossupressão (Auphan et al., 1995, Scheinman et al., 1995). Eles também suprimem a adesão celular, a marginação e migração, ativação dos macrófagos, apresentação de antígenos, expressão de receptores de células T, ativação dos linfócitos T, proliferação, diferenciação e função das células maduras, incluindo citotoxicidade e função das células B como a produção de anticorpos (Sternberg, 2001). Os GCs também têm a propriedade de induzir a apoptose de linfócitos e timócitos, mas estes efeitos podem ser secundários, pela inibição da produção de citocinas e fatores de proliferação (Sternberg, 2001).

Os hormônios da classe dos GCs também têm um importante papel no cérebro, além da função regulatória exercida no eixo HPA (Pariante, 2003). O hipocampo é uma região do cérebro que está relacionada com a formação de novas memórias. Essa região corresponde a uma das áreas do organismo com maior número de GCRs. Estudos sugerem uma forte correlação envolvendo elevadas concentrações de GCs circulantes e redução do volume hipocampal. Essa "atrofia" hipocampal pode estar relacionada com uma série de alterações psiconeuroimunoendrócrinas (Bremner and Narayan, 1998). Uma das hipóteses aventadas seria a de que altos níveis de glicocorticóides (cortisol), como os liberados em decorrência de situações de estresse, resultariam em dano hipocampal (Sapolsky, 2000b, Sapolsky, 1996). A exposição a elevadas concentrações de cortisol levaria à diminuição da arborização dendrítica e perda neuronal, o que cronicamente explicaria tais reduções neuroanatômicas (Bremner and Narayan, 1998, Bremner et al., 1999, Sapolsky, 2000a, Sapolsky et al., 2000).

Em contrapartida aos efeitos nocivos dos GCs no cérebro, um outro hormônio natural parece ter efeitos protetores: a dehidroepiandrosterona (DHEA), um pró-hormônio esteróide sintetizado a partir do colesterol. A molécula de colesterol é convertida em pregnenolona a partir de uma reação de clivagem de sua cadeia lateral em uma reação catalisada por uma enzima

mitocondrial dependente do citocromo P450 denominada Enzima de Clivagem da Cadeia Lateral do Colesterol (P450scc), também conhecida como Colesterol Desmolase ou CYP11A1. A pregnenolona é então convertida a DHEA pela enzima P450c17, responsável tanto pela hidroxilação da pregnenolona a 17-OH pregnenolona quanto pela sua conseqüente conversão a DHEA. A biosíntese de DHEA ocorre principalmente nas glândulas adrenais, mas também nas gônadas e no cérebro. A sulfatação do DHEA a sua forma mais estável e solúvel como éster sulfatado (DHEAS – sulfato de dehidroepiandrosterona) é catalisada pela enzima hidróxiesteróide sulfotransferase (HST), comumente denominada DHEA sulfotransferase. O DHEAS pode ser convertido novamente a DHEA pela enzima esteróide sulfatase (STS). Juntos, DHEA e DHEAS, frequentemente referidos como DHEA(S), representam os hormônios esteróides mais abundantes no organismo humano (revisado por Maninger et al., 2009).

A função mais antiga conhecida do DHEA(S) está relacionada com o seu papel no desenvolvimento de características sexuais secundárias, pois está envolvido no processo de síntese de estrógeno e testosterona. Com base em novas descobertas, o DHEA tem sido associado a outras propriedades, como as antidepressivas e neuroprotetoras (antagônicas aos GCs), sendo a relação cortisol/DHEA apontada como a melhor mensuração da ação relativa dos dois esteróides no cérebro (Maninger et al., 2009, Kalimi et al., 1994, Kaminska et al., 2000, Kimonides et al., 1998, Kimonides et al., 1999). Propriedades antiinflamatórias e imunomodulatórias do DHEA(S) também foram reportadas, como a inibição de citocinas TNF-α e IL-6 em modelos animais *in vivo* e em cultivos de células humanas *in vitro* (Kipper-Galperin et al., 1999, Chen and Parker, 2004, Iwasaki et al., 2004, Kimura et al., 1998, Straub et al., 1998).

Há muito sabe-se que as catecolaminas epinefrina (EPI) e norepinefrina (NEPI) são responsáveis por preparar o organismo para uma das respostas mais primitivas do mundo animal:

a resposta de “luta ou fuga”, relacionada ao estresse. As catecolaminas aumentam a contratibilidade e a velocidade de condução dos cardiomiócitos, levando ao aumento da freqüência cardíaca. As catecolaminas também promovem a dilatação brônquica, facilitando a respiração, além de promover a mobilização de reservas metabólicas do organismo (via lipólise e glicogenólise), provendo energia para órgãos e sistemas vitais (Flierl et al., 2008).

A ativação do SNS e a liberação de substâncias como as catecolaminas (EPI e NEPI), o peptídeo Y, opióides endógenos como a endorfina, além de adenosina e adenosina 5’-trifosfato, todos com efeitos diretos sobre a atividade de células imunes (Straub, 2004), fazem do SNS a segunda via de comunicação entre cérebro e o sistema imune. Segundo Wahle e colaboradores (Wahle et al., 2006), o papel fisiológico do SNS na geração de respostas imunes não foi, ainda, completamente elucidado, sendo a hipótese mais aceita a de que sua função esteja relacionada a um ajuste fino da magnitude e/ou duração da resposta imune. Embora não se tenha certeza sobre o seu papel imunológico, o SNS interage com tecidos linfóides e com células do sistema imune. Já foi demonstrado que: **a)** os tecidos linfóides são densamente inervados pelos SNS; **b)** neurotransmissores são liberados via nervos simpáticos diretamente nos tecidos linfóides; **c)** células do sistema imune expressam receptores adrenérgicos dos subtipos α e β (receptores aos quais se ligam EPI e NEPI), sendo o receptor tipo $\beta 2$ o mais prevalente (Kohm and Sanders, 2001), mas havendo presença também de receptores do tipo $\alpha 1$ (Kavelaars, 2002); e **d)** após a liberação de catecolaminas, há uma resposta significativa por parte das células do sistema imune (Straub, 2004).

Como as catecolaminas são produzidas na medula adrenal e esta se encontra intimamente ligada ao sistema nervoso, um conceito antigo definia esse conjunto como único responsável pela

produção, armazenamento e liberação das catecolaminas. Contudo, nas últimas décadas, evidências demonstraram que células do sistema imune (linfócitos e fagócitos) sintetizam e liberam neuropeptídeos, neurotransmissores e hormônios, tendo, portanto, funções adrenérgicas e colinérgicas (Flierl et al., 2008, Flierl et al., 2007) e tornaram ainda mais claro que essas substâncias são mediadores universais comuns que permitem a comunicação neuroimunoendócrina (Blalock, 2005). Por meio delas, os três sistemas envolvidos regulam positiva ou negativamente suas respostas, permitindo, portanto, que o organismo se adapte rapidamente a mudanças ou distúrbios internos (fisiológicos) e/ou externos (provenientes do ambiente) (Flierl et al., 2008)

Via comunicação direta por fibras nervosas simpáticas que inervam os tecidos linfóides (Straub, 2004), as catecolaminas foram descritas como sendo capazes de modular a proliferação linfocitária de camundongos (Swanson et al., 2001), a produção de citocinas de células T auxiliares (Th) de roedores (Sanders et al., 1997, Sanders and Straub, 2002) e de células mononucleares de sangue periférico humano (*peripheral blood mononuclear cells* – PBMCs) (Torres et al., 2005). Essas interações são facilitadas pela presença de receptores adrenérgicos em células do sistema imune. Estudos demonstraram a expressão desses receptores em linfócitos murinos (Sanders et al., 1997), em células NK de ratos (Peng et al., 2004), em macrófagos e neutrófilos de roedores (Flierl et al., 2007) e em PBMCs de humanos (Marino et al., 1999). Em um estudo *in vitro* que avaliava o perfil de produção de citocinas por linfócitos T humanos CD4+ e CD8+ por meio da quantificação das citocinas IFN-γ, TNF-α, IL-4 e IL-10 em sobrenadante de culturas estimuladas, cultivadas com EPI e NEPI, e pela avaliação das citocinas IFN-γ e IL-10 intracelulares, os autores mostraram claramente (em indivíduos saudáveis e com Artrite

Reumatóide) alterações no perfil de produção dessas citocinas, sugerindo que as catecolaminas influenciam no direcionamento das respostas imunológicas (Wahle et al., 2006).

A primeira evidência de que as catecolaminas poderiam ter origem diferente de tecidos nervosos ou endócrinos aconteceu a mais de uma década atrás, quando foi descrita a presença de catecolaminas intracelulares em linfócitos humanos (Bergquist et al., 1994). Essas catecolaminas eram secretadas e, de maneira autócrina, regulavam negativamente a proliferação linfocitária, diferenciação e apoptose, em camundongos e humanos (Bergquist et al., 1994, Josefsson et al., 1996). Estudos mais recentes demonstram a presença de toda uma "maquinaria" de enzimas, receptores e outras moléculas que permitem às células do sistema imune produzir, armazenar e secretar neurotransmissores como EPI, NEPI e dopamina. Esses dados demonstram que as células do sistema imune reagem tanto a substâncias produzidas na medula adrenal, quanto a substâncias produzidas pelas próprias células e/ou por células adjacentes (regulação autócrina e/ou parácrina) (Flierl et al., 2008).

Desde a comprovação da existência de relação entre o cérebro e o sistema imune, estudos foram desenvolvidos relatando como estados emocionais - dentre os quais o estresse e a depressão - podem afetar as respostas imunológicas em humanos e animais (Ader et al., 2001). Indivíduos com patologias como as supramencionadas têm uma qualidade de vida prejudicada e podem se tornar mais susceptíveis a doenças, pois, além de afetar as relações sociais, estes estados emocionais podem também comprometer a funcionalidade do sistema imune.

Sendo a mais importante causa de suicídios do mundo e custando mais de 30 bilhões de libras anuais somente no Reino Unido (UK) e nos Estados Unidos da América (EUA), a Depressão Maior (DM) é um transtorno psiquiátrico amplamente estudado em áreas clínicas e

científicas (Pariante, 2003). Essa condição é marcada por transtornos de humor como tristeza, inabilidade de ter prazer e falta de energia (Pariante, 2003) e freqüentes distúrbios de personalidade (Corruble et al., 1996). Ela também produz alterações fisiológicas importantes como mudanças na produção e secreção de hormônios, afetando diferentes sistemas, como o sistema imune. A Depressão Maior é conhecida por promover alterações no eixo HPA. Indivíduos com DM apresentam, geralmente, hiperatividade deste eixo, resultando em aumento dos níveis basais de cortisol e comprometimento do sistema de inibição da secreção deste hormônio (Hansen-Grant et al., 1998). A desregulação da secreção de cortisol e o comprometimento da regulação deste processo pelo *feedback* negativo mediado por GCs têm sido identificados como marcadores biológicos correlacionados à depressão e ansiedade (Burke et al., 2005, Holsboer et al., 1995). Além de alterações no eixo HPA, do ponto de vista imunológico, a DM está consistentemente associada a aumentos nas concentrações de citocinas pró-inflamatórias como IL-6 e TNF-α (Dantzer et al., 1999, Frommberger et al., 1997, Heuser, 1998, Holsboer and Barden, 1996, Maes et al., 1999), resultantes da resistência relativa aos efeitos dos GCs (Raison and Miller, 2003). Há indícios também de alterações na distribuição linfocitária e na expressão de moléculas de adesão, após administração de dexametasona (um potente GC sintético) em indivíduos com depressão resistente ao tratamento (Bauer et al., 2002) e evidências associando depressão a uma menor atividade de células NK e a uma menor capacidade de proliferação linfocitária após estímulo mitogênico inespecífico (Bauer et al., 1995b).

O estresse é uma condição que pode ser classificada de diferentes maneiras, dependendo de características que envolvem, principalmente, o agente estressor. Os agentes estressores são muito variáveis sob inúmeros aspectos, podendo diferir no tipo e na duração do evento estressor; na duração e na dimensão da resposta ao evento; bem como na maneira como cada indivíduo

assimila o evento estressante ao qual foi submetido, mesmo após ter respondido a ele (período de recuperação ou adaptação) (Delahanty et al., 2001b). Estressores traumatizantes severos como os que envolvem risco de vida, agressões e maus-tratos físicos e mentais, mesmo que de curta duração e sem repetição (uma única exposição), podem vir a gerar Transtorno do Estresse Pós-Traumático (TEPT) ou TEPT-*like*, assim denominado por compartilhar alguns mesmos sintomas clínicos de um quadro de TEPT, sem satisfazer todos os critérios requisitados para a caracterização da doença. Nesses transtornos psicológicos observa-se que reações emocionais ao evento traumático persistem por longos períodos após a ocorrência do mesmo, sugerindo que as respostas a tais traumas sejam resistentes à adaptação (Delahanty et al., 2001a).

Frente a uma situação física ou psicossocial adversa o indivíduo é forçado a se adaptar para manter sua sobrevivência. O processo ativo de adaptação do organismo a fim de que este se mantenha estável (em homeostasia) é chamado de Alostase. Quando a resposta alostática é excessiva ou ineficaz, o organismo desenvolve o que se chama de "peso alostático ou carga alostática". O acionamento repetitivo desses mecanismos adaptativos faz com que o organismo passe a funcionar em "estado alostático" e tais ações repetidas trazem conseqüências severas ao indivíduo (emocionais e fisiológicas) (McEwen, 2001, McEwen, 2002). Maus-tratos tais como o abuso e a negligência infantil (ANI) seriam exemplos de situações adversas passíveis de gerar estados de "carga alostática" em um organismo ainda vulnerável em seu desenvolvimento. Estudos envolvendo avaliações em humanos e em modelos experimentais mostram efeitos neuroendócrinos severos e permanentes, resultantes de eventos estressantes na infância (Coplan et al., 2001, Heim and Nemeroff, 2001, Meaney et al., 1989, Plotsky and Meaney, 1993). De fato, estudos demonstram alterações psíquicas em indivíduos que relatam exposição a este tipo de situação (Bremner et al., 1999, Bremner et al., 2003a, Ford and Kidd, 1998, Nemeroff et al.,

2006, Wright et al., 2003). Em particular, três estudos revisam a relação entre abuso infantil (particularmente abuso sexual e físico) e conseqüências neurobiológicas na vida adulta incluindo vulnerabilidade a desordens psicológicas como a depressão unipolar (Heim and Nemeroff, 2001, Teicher et al., 2006, Nemeroff et al., 2006). Contudo, poucos estudos investigaram os efeitos psicobiológicos gerados por situações de maus-tratos na infância relacionados à negligência (De Bellis, 2005).

Segundo a Organização Mundial da Saúde – OMS, maus-tratos na infância correspondem a um problema global com severas conseqüências na vida adulta. As vítimas apresentam maior predisposição à depressão; ao uso abusivo de drogas lícitas e ilícitas; à obesidade; além de apresentarem comportamentos sexuais de alto risco, aumentando a prevalência de doenças sexualmente transmissíveis e de gravidez indesejada. Além das conseqüências ao desenvolvimento social as conseqüências dos maus-tratos infantis também têm elevado impacto econômico a União. Hospitalização, medicação, acompanhamento da saúde mental e outras despesas de longa duração são frequentemente necessárias (WHO, 2010). No Brasil, segundo o Fundo das Nações Unidas para a Infância, estima-se que 18 mil crianças e adolescentes sejam violentados diariamente (ISPCAN, 2006).

Sabe-se que o estresse crônico está associado a um aumento da atividade do eixo hipotálamo-pituitária-adrenal (HPA), elevação da produção/secreção de cortisol e diminuição dos níveis de DHEA. Estudos também demonstram a ocorrência de hipercortisolemia em indivíduos com TEPT associado a diferentes eventos estressores, dentre os quais ANI (Putnam and Trickett, 1997, De Bellis et al., 1999b, De Bellis et al., 1999a, De Bellis, 2002b). A severidade do abuso também é correlacionada positivamente com o aumento do cortisol (Cicchetti and Rogosch, 2001). Contudo, a maioria destes estudos utiliza uma única amostra diária como medida para a

análise hormonal. Sabe-se que é falha a comparação de medidas únicas de cortisol entre diferentes indivíduos, devido a grande variabilidade diária de produção/secreção deste hormônio (efeito conhecido como ritmo circadiano), especialmente nos níveis matinais, e também entre os sexos dos participantes. Por isso, medidas da variação diurna do hormônio são mais fidedignas (Yehuda et al., 1996). Vários estudos que utilizam esse tipo de análise têm mostrado que adultos com TEPT e história prévia de ANI ou sobreviventes de guerras e holocaustos, apresentam um eixo HPA hiporesponsivo. Essa atividade reduzida do eixo HPA está caracterizada por baixas concentrações de cortisol basal (Yehuda et al., 2005, Rohleder et al., 2004, Heim et al., 2001), baixa resposta de cortisol ao acordar (Wessa et al., 2006, Rohleder et al., 2004) e aumento da capacidade de supressão da secreção de ACTH e cortisol após a administração de GCs (Yehuda et al., 2004c, Yehuda et al., 2006b). Em pacientes com ANI, essa alteração neuroendócrina estaria, provavelmente, relacionada com uma reprogramação do eixo HPA durante a infância, secundária à ativação crônica de altos níveis de cortisol devido a situações estressantes e traumáticas como o abuso sexual (Heim et al., 2001).

O estresse crônico está associado com uma importante desregulação neuroimunoendócrina. Em particular, foi demonstrado que cuidadores de pacientes com demência apresentam níveis mais elevados de cortisol salivar e linfócitos circulantes mais resistentes ao tratamento *in vitro* com glicocorticóides (Bauer et al., 2000b). Desta forma, além de causar imunossupressão, o estresse crônico altera a regulação linfocitária pelos glicocorticóides, trazendo conseqüências indesejadas para o sujeito. Por exemplo, esta desregulação neuroimunoendócrina pode contribuir para a etiologia ou curso clínico de doenças auto-imunes, cuja terapêutica usual ainda é o uso de glicocorticóides sintéticos. No entanto, existem evidências na literatura que sugerem que o TEPT está associado com uma maior sensibilidade dos receptores para glicocorticóides. Por exemplo,

foi demonstrado que pacientes com esse transtorno possuem células periféricas (Yehuda et al., 2004b, Rohleder et al., 2004) e eixo HPA (Yehuda et al., 2004a, Yehuda et al., 2006b, Yehuda et al., 1993) mais sensíveis a glicocorticóides, quando comparados a grupos controle. Um estudo com soldados veteranos de guerra com sintomas de TEPT verificou a ocorrência de maior número intracelular de glicorreceptores em células B, T e NK, quando comparadas a um grupo de voluntários saudáveis (Gotovac et al., 2003). Em particular, um trabalho (Yehuda et al., 2004b) demonstrou que as células mononucleares de pacientes com TEPT apresentam maior sensibilidade ao tratamento *in vitro* com dexametasona, fenômeno correlacionado à idade da exposição ao primeiro evento traumático. Contudo, o ensaio utilizado (supressão da produção de lisozima) não permite especificar qual população celular (linfócito ou monócito) foi avaliada.

Os glicocorticóides podem induzir a produção de citocinas Th2 em humanos (Ramirez et al., 1996, Galon et al., 2002). Sabe-se que o estresse crônico leva a uma alteração no padrão de citocinas produzidas pelos linfócitos T auxiliares. O aumento de cortisol e concomitante diminuição de DHEA induzidos pelo estresse podem produzir tais alterações. Em particular, evidências sugerem que o estresse crônico induz um aumento da produção de citocinas do padrão Th2 (IL-10, IL-4, TGF-β) e diminui as citocinas Th1 (IL-2, IFN-γ) (Biondi, 2001). Essa importante alteração poderia explicar a redução na imunidade celular, freqüentemente observada em indivíduos durante o estresse crônico.

Resultados envolvendo a resposta imune mediada por células, em pacientes com TEPT, são controversos. Um estudo (Altemus et al., 2003) verificou que um grupo de mulheres com história de ANI e sintomas de TEPT mostrava um aumento da resposta imune celular. Esses achados encontram-se alinhados com resultados apresentados em trabalhos anteriores envolvendo ativação imunológica em TEPT (Sabioncello et al., 2000, Watson et al., 1993, Boscarino and

Chang, 1999, Laudenslager et al., 1998, Wilson et al., 1999). Relatos apontam também para um possível aumento na produção de citocinas pró-inflamatórias (IL-6, IL-1 e TNF-α) e uma diminuição na produção de IFN-γ (Spivak et al., 1997, Maes et al., 1999, Dekaris et al., 1993), sugerindo uma ativação crônica da resposta inflamatória em pacientes com TEPT (Rohleder et al., 2004). Todavia, resultados contrários também são encontrados na literatura (Kawamura et al., 2001). Embora haja muitas evidências de um aumento na atividade imune celular e na produção de citocinas pró-inflamatórias em casos de TEPT, não existem estudos que tenham verificado o padrão de proliferação de células envolvidas na resposta imune e sua conseqüente secreção de citocinas de perfil Th1/Th2 em indivíduos adultos, com DM e sintomas de TEPT gerados a partir de ANI.

Frente ao exposto, a presente tese tem por objetivo contribuir agregando conhecimento a uma importante questão de pesquisa levantada no campo da traumatologia desenvolvimental e com impactos sociais severos (De Bellis, 2002b, De Bellis, 2005), que procura responder se a negligência e o abuso na infância podem ser estressores crônicos capazes de alterar sistemas biológicos e afetar o desenvolvimento neuropsicológico. Esta tese pretende contribuir para a compreensão das interações neuroimunoendócrinos correlacionadas a situações estressantes traumáticas, como o ANI, em um grupo de mulheres adultas com DM, patologia frequentemente encontrada em associação a quadros de TEPT. Para tanto, foram avaliados parâmetros hormonais e imunológicos, além do monitoramento psicológico e cognitivo em três grupos de mulheres adultas divididas pela presença ou não de distúrbios psiquiátricos. Mais especificamente, avaliaram-se os níveis salivares de cortisol e de DHEA; a mitogênese induzida de linfócitos T de sangue periférico; a sensibilidade de linfócitos T estimulados aos efeitos de moduladores sintéticos (dexametasona) e naturais (epinefrina e sulfato de dehidroepiandrosterona); a secreção

de citocinas de perfis Th1/Th2 (IL-2, IL-4, IL-6, IL-10, TNF-a, IFN-g) por células mononucleares estimuladas com fitohemaglutinina; e os níveis plasmáticos de BDNF, além de TNF-a e seus receptores solúveis (sTNFR1 e sTNFR2).

2. DESENVOLVIMENTO

2.1. ARTIGO CIENTÍFICO

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NEUROIMMUNOENDOCRINE INTERACTIONS IN PATIENTS WITH RECURRENT MAJOR DEPRESSION, INCREASED EARLY LIFE STRESS AND LONG-STANDING PTSD SYMPTOMS

Rodrigo Pestana Lopes [a,f], Rodrigo Grassi-Oliveira [c], Lettícia R. de Almeida [a],

Lilian Milnitsky Stein [c], Clarice Luz [d], Antonio L. Teixeira [e] and Moisés E. Bauer [a,b]

[a]Institute of Biomedical Research, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre, Brazil;

[b]Faculty of Biosciences, PUCRS, Porto Alegre, Brazil;

[c]Faculty of Psychology, PUCRS, Porto Alegre, Brazil;

[d]LabVitrus, Porto Alegre, Brazil;

[e]Department of Internal Medicine, School of Medicine, Federal University of Minas Gerais (UFMG), Belo Horizonte, Brazil;

[f]BD Biosciences, São Paulo, Brazil.

Running head: Neuroimmunoendocrine interactions in major depression

Key words: depression, Th1/Th2 cytokines, glucocorticoids, early life stress, child abuse, trauma

Corresponding author: Dr. Moisés E. Bauer, Instituto de Pesquisas Biomédicas, Av. Ipiranga 6690, 2º andar. P.O. Box 1429. Porto Alegre, RS 90.610-000, Brazil. Email: mebauer@pucrs.br

ABSTRACT

Background: Traumatic events experienced in childhood may lead to psychiatric diseases in adult life, including major depressive disorder (MDD). It is still obscure to what extent early life stress is associated with biological relevant changes in MDD. **Objective:** Here, we investigated both neuroendocrine and immunological correlates in recurrent MDD with early life stress and current PTSD symptoms. **Methods:** Thirty-eight female MDD patients with or without childhood trauma and 15 healthy controls took part in this study. Salivary cortisol and dehydroepiandrosterone sulphate (DHEAS) were assessed by radioimmunoassays. Peripheral blood mononuclear cells (PBMCs) were isolated and T-cell proliferation and cellular sensitivity to steroids and DHEAS were evaluated by colorimetric assays. Th1/Th2 cytokines were assessed by cytometric bead arrays. **Results:** MDD patients with or without previous trauma had similarly lower salivary cortisol and DHEAS in parallel with blunted T-cell proliferation. PBMCs of depressives were significantly less sensitive to dexamethasone or epinephrine than controls. PBMCs of MDD patients produced significantly lower IL-2, IL-4 and TNF-α levels when compared to healthy controls. **Conclusion:** We conclude that a history of early life stress did not modify the blunted neuroendocrine and immunological alterations presented by recurrent depressed patients.

Introduction

A history of early life stress (ELS), such as childhood maltreatment (abuse and neglect) has been suggested to mediate vulnerability to subsequent affective disorders including Post-Traumatic Stress Disorder (PTSD) and major depression (Heim and Nemeroff, 2001). More recently, several studies have related childhood trauma with neurobiological consequences in adult life (Grassi-Oliveira et al., 2008, Teicher et al., 2006, Widom et al., 2007, Enoch, 2010), in a process hypothesized to be mediated by the hypothalamic-pituitary-adrenal (HPA) axis. More specifically, the influence of higher childhood stress load on the appearance of later psychopathologies has been linked to the particular sensitivity of the developing brain and hormonal system (Charmandari et al., 2003). Moreover, ELS has been associated to increased cortisol secretion in response to a laboratory stressor during adulthood (Heim et al., 2000).

Major Depressive Disorder (MDD) has repeatedly been associated with neuroendocrine and immunological changes. Of particular note, it has been shown that MDD patients had increased cortisol levels and reduced HPA axis feedback control (Burke et al., 2005, Holsboer et al., 1995, Yehuda et al., 2005). Moreover, MDD has also been linked to reduced glucocorticoid-induced immunoregulation (Bauer et al., 2003, Raison and Miller, 2003) and to low-grade inflammatory responses. Indeed, MDD has been related with higher levels of soluble tumor necrosis factor (TNF)-α receptors (sTNFR1 and sTNFR2) (Grassi-Oliveira et al., 2009) as well as elevated pro-inflammatory cytokines and chemokines such as MCP-1, IL-1β, IL-6, IL-8, IFN-γ and TNF- α (Raison et al., 2006, Yang et al., 2007, Grassi-Oliveira et al., 2009, Simon et al., 2008).

In contrast to depression, PTSD patients usually show reduced HPA activity associated with low cortisol basal levels (Bierer et al., 2006, Carpenter et al., 2007, Heim et al., 2001,

Meewisse et al., 2007), low cortisol awakening response (Yehuda, 2006, Rohleder et al., 2004, Wessa et al., 2006) and increased sensitivity to administered glucocorticoids (Rohleder et al., 2004, Yehuda et al., 2004b, Griffin et al., 2005) when compared to healthy controls. In addition to depressive symptoms, it has also been hypothesized that PTSD might influence the relationship between ELS and the HPA axis function as well (de Kloet et al., 2007). For example, women with history of sexual abuse and current PTSD have shown increased urinary cortisol excretion and increased glucocorticoid feedback sensitivity (Lemieux and Coe, 1995, Stein et al., 1997). In line with previous data reported in depression, PTSD has also been linked with chronic low-grade inflammation (Gill et al., 2009, Bauer et al., 2010).

Stress and depression may also influence other adrenal steroids such as dehydroepiandrosterone (DHEA) and its sulfated form (DHEAS). These steroids represent the most abundant steroid hormones in the human body. However, DHEA has received considerably less scientific attention in psychiatric conditions than cortisol. Serum DHEA assessments in MDD have revealed elevated (Maayan et al., 2000, Heuser et al., 1998), unchanged (Young et al., 2002) or reduced hormonal levels (Goodyer et al., 1996, Herbert et al., 1996, Scott et al., 1999) when compared to controls. It has been suggested that DHEA may antagonize various stress-related effects of glucocorticoids on peripheral tissues as well as in the hippocampus (Kaminska et al., 2000, Kimonides et al., 1998, Kalimi et al., 1994). The measurement of isolated hormones is an oversimplification and the assessment of molar concentrations (cortisol/DHEA ratio) may thus improve the understanding of functional glucocorticoid actions.

Here, we sought to investigate the effects of a history of ELS and PTSD symptoms on several neuroendocrine and immunological components of women with recurrent MDD compared to age-matched controls. In particular, we evaluated the (a) HPA axis function across the day (corti-

sol and DHEAS measurements), (b) T-cell proliferation as an index of cell-mediated immunity, (c) investigated the peripheral sensitivity to adrenal hormones, and finally (d) measured various secreted Th1/Th2 cytokines implicated with cell-mediated immunity.

Materials and Methods

Subjects and Clinical Assessment

Sixty recurrent MDD outpatients (all female subjects, 20 – 55 years) from an affective disorder program of a public general hospital (Hospital Presidente Vargas, Porto Alegre, Brazil) were initially interviewed by two well-trained psychiatrists with the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) to confirm the diagnosis of recurrent MDD. Low social status was evaluated by Hollingshead's Index of Social Status (Cirino et al., 2002). Severity of depressive symptoms was evaluated by Beck Depression Inventory (BDI) (Beck et al., 1961, Gorenstein and Andrade, 1996). Post-traumatic stress disorder (PTSD) symptoms were investigated by PTSD Checklist – Civilian Version (PCL-C) (Berger et al., 2004a, Lang et al., 2003) and childhood maltreatment (CMT) assessed by Childhood Trauma Questionnaire (CTQ) (Grassi-Oliveira et al., 2006a, Bernstein et al., 2003). All patients have been using a stable dosage of antidepressant monotherapy (SSRI or Tryciclics) for at least 3 months prior to the study and have been suffering from recurrent MDD for about 10 years. Exclusion criteria were: Axis I comorbidities, severe or unstable clinical illness or illness associated with reports on abnormal immunological parameters, body mass index (BMI) above 30 kg/m^2, neurological disorder, psychotic symptoms or any psychoactive substance (including alcohol) used in the last 30 days (excepting nicotine, caffeine and antidepressants). Twenty-two outpatients (mean age ± SD, 39.73 ± 10.12 yrs) with recurrent major depression and without history of childhood maltreatment (MDD

group); and 16 outpatients (41.06 ± 6.46 yrs) with recurrent major depression, actual severe PTSD symptoms (PCL-C > 50) and history of childhood maltreatment (CMT group) were enrolled in this study.

Ninety-four healthy controls (hospital employees, all females, 20–50 yrs), matched with patients by age, social status and BMI, were initially screened and evaluated with SCID-I, Hollingshead's Index, BDI, PCL-C and CTQ. Exclusion criteria were: any past or current Axis I disorder, any history of childhood maltreatment, severe or unstable clinical illness or illness associated with reports on abnormal immunological parameters, body mass index (BMI) above 30 kg/m^2, neurological disorder, psychotic symptoms or any psychoactive substance (including alcohol) used in the last 30 days (excepting nicotine and caffeine). Due to the rigorous exclusion criteria adopted, the majority of subjects were not included in the study. The control group (CTRL) corresponded to 15 healthy subjects (37.07 ± 5.64 yrs).

The assessment was performed early in the morning (07 a.m.) and at least four days after menstruation in all subjects without amenorrhea. After being provided with a complete description of the study, each participant signed a consent form and proceeded to saliva and blood collections. The current research was approved by Pontifical Catholic University of Rio Grande do Sul (PUCRS) Scientific and Ethics Committees.

Saliva collection and endocrine evaluation

Participants were asked to follow their normal daily routine and collect three saliva samples with the help of cotton rolls at 08 a.m., 12 p.m. and 08 p.m., always before meals and venipuncture. Sampling was performed across the day to assess some aspects of circadian pattern (Bauer et al., 2000a, Luz et al., 2003). All samples were collected within one month of the study to avoid

seasonal variation in the HPA axis function. Upon arrival in the laboratory, the samples were centrifuged and frozen at -20°C. Salivary cortisol (08 a.m., 12 p.m. and 08 p.m.) and DHEAS (08 a.m.) samples were analyzed in duplicates by radioimmunoassay (DPC Medlab, São Paulo, Brazil). The sensitivity of this assay was estimated in 0.1nM. The intra- and inter-assay coefficients of variation were less than 10%. Results from each of the sampling times were expressed in nM.

Collection of peripheral blood and isolation of mononuclear cells

Twenty milliliters of peripheral blood were collected by venipuncture into lithium-heparin tubes (BD Preanalytical Systems) in the morning (between 8-9 a.m.). Samples were always collected at the same time of day to minimize circadian variations. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient (Ficoll-Hypaque, Sigma) under centrifugation for 30 min at 900 g. Cells were counted by means of microscopy (100 x) and viability always exceeded 95%, as judged from their ability to exclude Trypan Blue (Sigma). PBMCs were resuspended in complete culture medium (RPMI-1640, supplemented with 0.5% gentamicine, 1% glutamine, 1% hepes, 0.1% fungizone, and 10% fetal calf serum; all from Sigma) and adjusted to yield a final working concentration of $3x10^6$ cells/mL.

Th1/Th2 cytokine quantification

PBMCs were cultured in triplicates in flat bottomed 96-well microplates (BD Biosciences) in a final concentration of $1.5x10^5$ cells/200 µL/well in complete culture medium containing 1% of the mitogenic lectin phytohemagglutinin (PHA; from Gibco) for T-cell polyclonal activation. Cells were incubated for 96h, at 37ºC and in a 5% CO_2 atmosphere. After incubation, 100µL of each well's supernatant was gently aspirated and stored frozen (-80°C) until analysis. The Cytometric Bead Array (CBA) system was chosen for this evaluation due to its property to simulta-

neously measure multiple soluble proteins in a single sample (50μL) using flow cytometry. CBA Human Th1/Th2 Cytokine Kit II (BD Biosciences) was used to quantitatively measure Interleukin-2 (IL-2), Interleukin-4 (IL-4), Interleukin-6 (IL-6), Interleukin-10 (IL-10), Tumor Necrosis Factor-α (TNF-α), and Interferon-γ (IFN-γ). Procedures followed manufacturer's recommendations. Data was acquired in a FACSCalibur flow cytometer equipped with a 488nm laser and CellQuest Pro software (BD Biosciences). The instrument has been checked for sensitivity and overall performance with 3-color CaliBRITE particles (BD Biosciences) and FACSComp software (BD Biosciences). Sample results (conversion of fluorescence intensities to concentration) were generated using FCAP Array v1.0.1 software (BD Biosciences / Soft Flow Hungary Ltd.) and are expressed as pg/mL. According to the manufacturer, the theoretical detection limits for each analyte were: 2.6pg/mL (IL-2 and IL-4); 3.0pg/mL (IL-6); 2.8pg/mL (IL-10 and TNF-α); and 7.1pg/mL (IFN-γ). A five-parameter logistic (5-PL) equation was used as the mathematical fitting model chosen to generate the standard curves of all measured cytokines and the curve correlation index (R^2) for each standard curve was: 99.99% (IL-2); 99.97% (IL-4); 99.90% (IL-6); 99.99% (IFN-γ); 99.90% (TNF-α) and 99.79% (IL-10).

Lymphocyte proliferation/viability assays

Mitogen-induced T-cell proliferation was evaluated as an index of cell-mediated immunity. Peripheral sensitivity to hormones was estimated by functional assays developed to measure the ability of steroids to suppress T-cell proliferation *in vitro*. PBMCs were cultured in flat bottomed 96-well microplates (BD Biosciences) in a final concentration of $1.5x10^5$ cells/200 μL/well in complete culture medium, for 96h, at 37ºC and in a 5% CO_2 atmosphere. Polyclonal stimulation by the selective T-cell mitogen PHA was performed in triplicates to yield three optimal concen-

trations (0.5, 1 and 2%). In non-stimulated cultures (PHA 0%), the mitogen was replaced by complete culture medium. Dexamethasone (DEX, a synthetic selective glucocorticoid receptor agonist), epinephrine (EPI) and DHEAS were separately added in duplicates (50μL/well; all water-soluble substances from Sigma) to mitogen-stimulated lymphocyte cultures (PHA 1%). Due to the varied cellularity presented in the blood samples of each evaluated individual, some subjects did not have enough isolated PBMCs for all proposed cellular investigations. Sample size for each evaluation is displayed on the legend of the correspondent graph.

Proliferative responses were estimated by a MTT (Sigma) colorimetric assay that correlates with the number of viable cells as previously described and routinely performed in our laboratory (Borowski et al., 2007, Lopes et al., 2006, Pillat et al., 2009, Ribeiro et al., 2007). The optical density (OD) was determined using an ELISA plate reader at a wavelength of 570 and 630 nm (Biorad). Proliferation/viability was expressed as the OD of stimulated minus OD of non-stimulated cultures. Cellular sensitivity data is represented by basal proliferation, with 100% (basal) corresponding to PHA 1% proliferation without steroids.

Responsiveness to adrenal products

As previously described (Lopes et al., 2007), steroid responders and non-responders were selected to each one of the adrenal hormones studied in healthy controls. Briefly, PBMCs were cultured with 1% PHA and varied concentrations of DEX, EPI or DHEAS (as mentioned in the previous section). The area under the curve (AUC) for each control subject was then calculated by the trapezoidal rule and the medians were calculated (DEX = 348.5 M; EPI = 444.4 M; DHEAS = 437.2 M). The same AUC determination was performed individually for each patient (MDD and CMT groups). Patients with an AUC higher than the median AUC of the controls

were classified as non-responders for that specific hormone. Patients with an AUC lower than these values were considered to be sensitive to these hormones *in vitro*, as their dose-response curve indicated lower proliferation percentages, and were thus classified as responders.

Statistical analysis

All variables were tested for homogeneity of variances and normality of distribution by means of the Levene and Kolmogorov-Smirnov tests, respectively. Group mean differences were assessed by means of the one-way analysis of variance (ANOVA). Multiple comparisons among group mean differences were checked with the Tukey's honestly significant difference (HSD) post-hoc test. Cortisol circadian rhythm and proliferation data were analyzed by repeated measures ANOVA that included one between-subjects variable (groups) and one within-subjects variables (cortisol levels; mitogen, DEX, DHEAS or EPI concentrations). Cytokine data were analyzed by Kruskal-Wallis one-way analysis of variance. Multiple comparisons among levels/concentrations were checked with Tukey's HSD post hoc test. Statistical interactions between group distributions were compared by means of the chi-square ($\chi 2$) test. Statistical analyses were performed using the Statistical Package for Social Sciences, SPSS Statistics 17.0 software (SPSS Inc.). Graphs and AUC determinations were generated with GraphPad PRISM 5.0 (GraphPad Software Inc.). The significance level was set at $\alpha = 0.05$ (two-tailed).

Results

Socio-demographic and clinical data

The demographic, psychosocial and clinical characteristics of samples are shown in Table 1. All groups were highly homogeneous regarding age, education, income and Body Mass Index

(BMI). There was also homogeneity in severity of depression, illness duration and time of anti-depressant treatment among patients (MDD and CMT groups).

Salivary cortisol and DHEAS levels

Salivary cortisol was evaluated as a key biological marker related with stress and depression. The cortisol levels differed significantly over the three sampling times in all groups, $F(2,84) = 277.07$, $p<0.001$, presenting a regular circadian rhythm (peak levels in the morning and nadir in the evening). Cortisol levels differed significantly among groups, $F(2,42) = 18.27$, $p<0.001$. Post hoc analyses revealed reduced cortisol levels in both depressed groups when compared to control (Fig. 1A). In agreement with these results, the MDD (39.58 ± 3.71 nM/h) and CMT (46.12 ± 3.99 nM/h) groups had similar decreased integrated (AUC) cortisol levels compared to controls (68.58 ± 5.17 nM/h), $F(2,45) = 12.21$, $p<0.0001$. DHEAS was also assessed as an important marker of adrenal function and because of its property to antagonize many GC-related changes. Both depressed groups had also significantly reduced DHEAS levels when compared to controls (Fig. 1B). However, cortisol/DHEAS ratio did not differ among groups (Fig. 1C).

T lymphocyte proliferation/viability and cellular sensitivity to hormones

We evaluated the mitogen-induced T-cell proliferation/viability *in vitro* as an index of cell-mediated immunity. Mitogenic polyclonal stimulation yielded significant T-cell proliferation observed by its capacity to increase the number of viable cells in all tested concentrations (0.5, 1 and 2%) when compared to non-stimulated cultures, $F(3,123) = 163.28$, $p<0.001$. Non-stimulated proliferation did not differ among groups ($p>0.05$) (mean ± SE; CTRL = 0.21 ± 0.03 OD, MDD = 0.22 ± 0.03 OD, CMT = 0.17 ± 0.02 OD). However, both MDD and CMT groups had significantly blunted T-cell proliferation ($p<0.001$) when compared to controls (Fig. 2).

It has largely been shown that glucocorticoids and catecholamines are able to modulate various immune functions, including lymphocyte proliferation. The PBMCs sensitivities to hormones were estimated by functional assays developed to measure the ability of hormones to suppress T-cell proliferation *in vitro*. DEX, DHEAS and EPI produced dose-dependent suppression of T-cell proliferation in all groups investigated (Fig. 3), ($p<0.001$). It was observed that PBMCs of MDD and CMT groups were similarly less sensitive to the variation of DEX concentrations (dose-response curve) when compared to controls (MDD, $p<0.05$; CMT, $p<0.01$). Moreover, PBMCs of CMT patients were less sensitive to the variation of EPI treatment *in vitro* than controls ($p<0.05$). Even though statistical interactions between groups were identified when comparing some isolated concentrations of DHEAS, no differences were observed in cellular sensitivities to the overall variation of DHEAS treatment between groups.

We also investigated the frequency of *in vitro* hormone non-responders within each group of patients when compared to controls. Within the MDD group, we found higher frequencies of non-respondent individuals following DEX (16 non-responders vs 2 responders; $\chi2 = 10.9$, d.f. = 1, $p = 0.001$) or EPI (12 non-responders vs. 3 responders; $\chi2 = 5.4$, d.f. = 1, $p = 0.02$) but not DHEAS treatment (5 non-responders vs. 6 responders; $\chi2 = 0.9$, d.f. = 1, $p = 0.76$). The CMT group had similarly higher frequencies of steroid non-responders in all three treatments when compared to controls: DEX (11 non-responders vs. 1 responder; $\chi2 = 8.3$, d.f. = 1, $p = 0.004$), EPI (10 non-responders vs. 2 responders; $\chi2 = 5.3$, d.f. = 1, $p < 0.02$) and DHEAS (9 non-responders vs. 2 responders; $\chi2 = 4.5$, d.f. = 1, $p = 0.04$).

Th1/Th2 cytokines

Table 2 shows supernatant levels of Th1/Th2 cytokines assessed by flow cytometry. MDD and CMT groups had significantly lower IL-2 and IL-4 levels when compared to healthy controls ($p<0.02$). In addition, cells of CMT group produced significantly less TNF-α than cells of MDD or control groups ($p<0.05$). There were no differences in IL-6, IL-10 and IFN-γ levels among groups ($p>0.05$).

Clinical correlates of endocrine and immune variables

We first assessed the clinical correlates of endocrine changes reported here. BDI (r = -0.37, p=0.01) or PCL-C scores (r = -0.36, p=0.01) were found negatively related to AUC cortisol levels or to morning DHEAS (BDI, r = -0.29, p<0.05; PCL-C, r = -0.42, p=0.001). The remaining clinical variables were not found correlated to adrenal hormones.

Zero-order analyses also revealed significant correlations with depression or PTSD scores with mitogen-induced proliferation: BDI (PHA 1%, r = -0.45, p=0.002) and PCL-C (PHA 1%, r = -0.48, p=0.001). To determine whether illness characteristics were predictive of T-cell proliferation, stepwise multiple regression analyses were employed using BDI scores, PTSD scores and morning (8 a.m.) cortisol as predictors. During the regression models there was no evidence of high colinearity between selected variables, and all VIF values were < 2. Cortisol entered in the equation as only predictor variable for PHA 1% ($R^2 = 0.35$) and PHA 2% ($R^2 = 0.28$). In addition, cortisol ($R^2 = 0.28$) or BDI ($R^2 = 0.35$) predicted independently the proliferative responses (PHA 0.5%). There were no significant correlations between sociodemographic (including age and sex) and clinical variables, cytokines and sensitivity to adrenal hormones.

Discussion

This study reported the neuroendocrine and immunological correlates in female patients with recurrent MDD, current PTSD symptoms and history of ELS. Briefly, depressed patients with or without ELS had reduced HPA axis activity as shown by significant lower salivary cortisol and DHEAS in parallel with blunted T-cell proliferation and lower cytokine levels than controls. Peripheral lymphocytes of depressives were similarly more resistant to DEX or EPI modulation than controls. In other words, PBMCs required a higher hormonal concentration to suppress lymphocyte proliferation *in vitro*. To our knowledge, this is the first study addressing simultaneously various endocrine and immunologic variables implicated with depression and ELS on adult women with long-standing PTSD symptoms.

The assessment of cortisol levels across the day provides more reliable information regarding the HPA axis functional activity. In this study, all depressed patients investigated (with and without ELS) had significantly reduced cortisol levels across the day when compared to controls. Hypocortisolism is a well defined finding of PTSD patients and has been associated with low basal cortisol levels, increased cortisol suppression after *in vivo* DEX administration and reduced awakening cortisol response (Bierer et al., 2006, Griffin et al., 2005, Rohleder et al., 2004, Wessa et al., 2006, Yehuda et al., 2004c, Yehuda, 2006). In contrast to the hypocortisolism observed in PTSD, the MDD has been mostly associated with activation of the HPA axis. However, discrepant results have been produced in the literature. In a study addressing the effects of psychotic symptoms in MDD, Keller and colleagues have shown that while psychotic depressed patients had increased evening cortisol secretion when compared to control subjects, depressed patients without psychosis presented no hormonal raises when compared to the same control group of individuals (Keller et al., 2006). Moreover, a study evaluating within-person variation of salivary cortisol levels over a 6-day period did not find clear evidence for hypercortisolism in 47 MDD

outpatients when compared to 39 healthy controls (Peeters et al., 2004). It has been suggested that since most studies reporting high cortisol levels in depression have evaluated hospitalized subjects and not outpatients, this could be a trend generated by the environment and that an erratic secretion of cortisol may be a more accurate characteristic of HPA dysregulation in MDD individuals. It should be noted that all MDD subjects enrolled in our study were outpatients and had chronic depressive illness, with average 10 years of illness duration. Thus, these patients may have been being exposed to higher levels of cortisol at disease onset, but after 10 years, HPA axis may be hyporeactive. We did not find any association with ELS, although the effects of ELS on the HPA axis could be masked by the chronicity of current illness. Therefore ELS-related effects could be only observed at disease onset.

We also measured salivary DHEAS levels as an important marker of adrenal function. Here, we observed that recurrent MDD patients (with or without PTSD symptoms) had lower DHEAS levels when compared to controls. However, current literature has produced discrepant results. There are reports of lower (Boscarino, 2004), elevated (Spivak et al., 2000, Sondergaard et al., 2002, Kellner et al., 2010) and unchanged (Rasmusson et al., 2004, Yehuda et al., 2006a, Olff et al., 2006) DHEAS concentrations as compared to healthy controls. DHEAS and its metabolites have been reported to have several protective biological actions including neuroprotection, neurite growth, and antagonistic effects on glucocorticoids (Maninger et al., 2009). Lower DHEAS levels in depression would be thus implicated in less protective physiological effects. The determinations of molar cortisol/DHEAS concentrations are thus more informative. Decreased cortisol/DHEAS ratio has already been associated with superior coping with the negative effects of acute stress in a healthy man population (Morgan et al., 2004) while elevated cortisol/DHEAS ratio was associated with increased all-cause disease mortality in a study with war

veterans (Boscarino, 2008). Here, no differences in cortisol/DHEAS ratios were observed between groups, indicating a positive finding of the functional steroid signaling in peripheral tissues.

The mitogen-induced T-cell proliferation/viability was evaluated here as an index of non-specific cell-mediated immunity. Depressed groups (MDD and CMT) had reduced mitogen-induced lymphocyte proliferation when compared to controls. Although lower non-specific lymphocyte proliferation had previously been reported in MDD (Bauer et al., 1995a), there is limited information regarding the impact of ELS on cell-mediated immune function. In line with the reduced *in vitro* proliferative responses, MDD groups presented lower cellular secretion of IL-2 and IL-4 when compared to controls. Of important note, the reduced IL-2 production could be interpreted as underlying mechanism for blunted T-cell responses. Major depression and PTSD have been associated with higher plasma levels of pro-inflammatory mediators such as acute phase proteins and cytokines (Raison et al., 2006, Yang et al., 2007, Grassi-Oliveira et al., 2009, Simon et al., 2008). However, little is known about secreted cytokine profiles in depression and discrepancies could be explained by methodological differences (i.e. plasma/serum vs. supernatant). A brief report demonstrated reduced Th1 (IFN-γ) and Th2 (IL-4) cytokine secretion by PHA-stimulated whole blood cells in a group of men with past PTSD (Kawamura et al., 2001). Future studies should address the clinical significance of this blunted cell-mediated immunity in depression.

The measurement of peripheral hormones may not be sufficient to finally determine the functional hormonal action in target tissues. Therefore, to further examine the cross talk between peripheral hormones and immune system, we also investigated the lymphocyte sensitivity to low concentrations of synthetic (DEX) and natural occurring adrenal hormones (EPI and DHEAS).

Here, we compared for this first time the immunoregulatory actions of EPI and DHEAS in cells of depressed patients with and without PTSD symptoms. Cells from depressed patients (MDD or CMT) were similarly less sensitive to DEX and EPI-related immunomodulation *in vitro* than controls. These results are in accordance to previous work on MDD (Bauer et al., 2003, Raison and Miller, 2003) but contrast previous and scarce data with PTSD patients. One particular study with a veteran population of men with PTSD has reported increased PBMC sensitivity to DEX, using a lysozyme activity assay (Yehuda et al., 2004b). Regarding the immunoregulatory actions of DHEAS, even though our results could not suggest a specific correlation between the cellular effects of this hormone and the clinical conditions here investigated, our findings suggest a possible relation of ELS with an increase on peripheral resistance to this hormone. Data presented in this work indicate an increased frequency of individuals with recurrent depression, current PTSD symptoms and history of ELS who needs higher concentrations of DHEAS to modulate polyclonal activated T lymphocytes when compared to depressive patients without PTSD symptoms or ELS experiences.

There are some limitations in this study to be discussed. First, the cross-sectional design precludes causal inferences. Second, participants were suffering from a recurrent MDD and were medicated, thus antidepressants as well as a chronic condition could be confounding variables in this study. The stringent inclusion/exclusion criteria have limited the sample sizes but yielded strictly healthy control subjects. Replication with large samples and longitudinal follow-up will be needed to overcome these limitations.

Data presented in this study suggest a low grade HPA activity in recurrent major depressed women undergoing pharmacological antidepressant treatment and that the long-standing

PTSD symptoms due to childhood maltreatment have no significant impact on endocrine and immunologic parameters in this sample.

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Table 1.

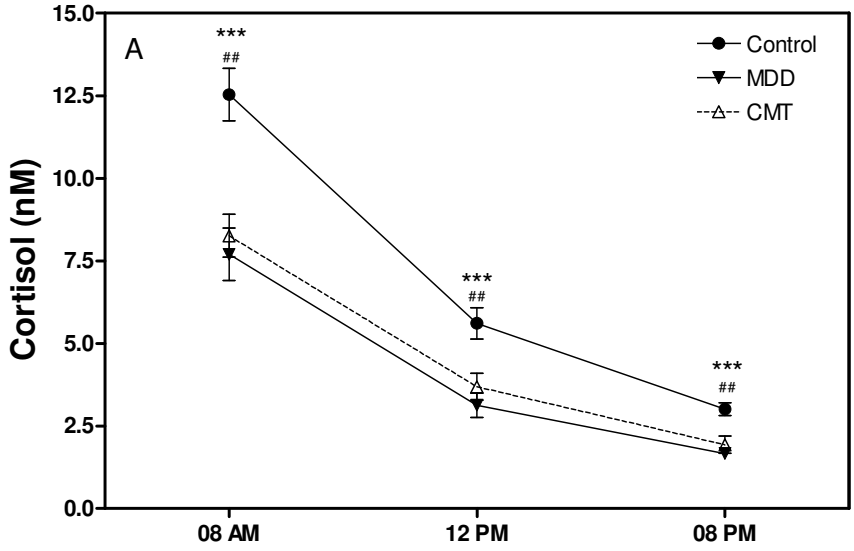
	MDD (n=22)	*CMT (n=16)*	*Control (n=15)*	*P*	*Post Hoc*
Age (years)	39.73 ± 10.12	41.06 ± 6.46	37.07 ± 5.64	0.38	NS
Education (years)	3.24 ± 1.84	3.07 ± 1.60	3.27 ± 0.96	0.93	NS
Income (US$/month)	460.96 ± 218.26	426.25 ± 193.15	495.00 ± 148.94	0.64	NS
Body Mass Index	26.65 ± 2.27	26.18 ± 2.25	25.37 ± 2.77	0.36	NS
Depression severity (BDI score)	20.45 ± 7.49	31.00 ± 10.55	4.53 ± 3.91	**< 0.001**	MDD=CMT > C
PTSD-like symptoms (PCL-C scores)	50.10 ± 14.06	63.44 ± 8.46	22.80 ± 5.23	**< 0.001**	CMT>MDD> C
Childhood Maltreatment (CTQ)	49.05 ± 16.52	65.31 ± 16.72	40.67 ± 11.02	**< 0.001**	MDD<CMT > C
Illness Duration (years)	9.69 ± 3.98	13.57 ± 6.52	---	0.06	NS
Treatment Time (years)	6.88 ± 3.34	8.36 ± 2.92	---	0.21	NS
Antidepressant					
Tricyclic	41.2%	52.9%	---		NS
SSRI	41.2%	41.2%	---		NS
Other	17.6%	5.9%	---		NS

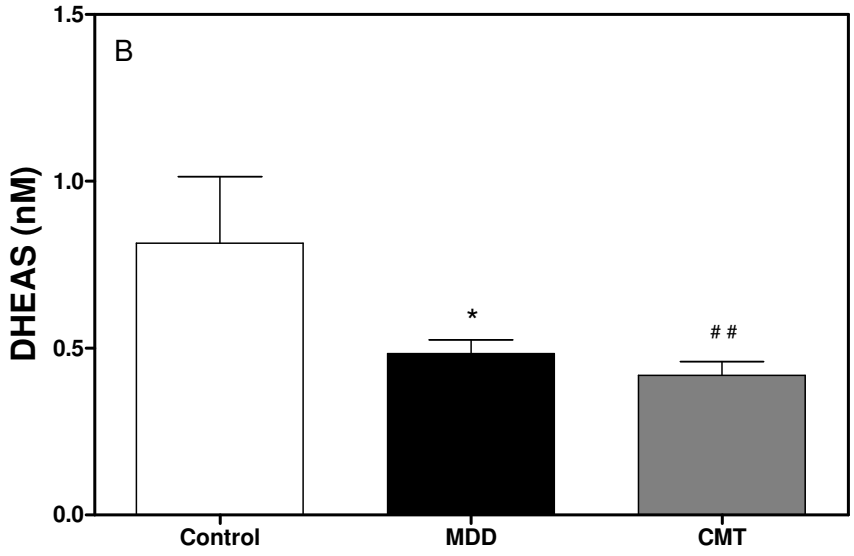
MDD = Major Depressive Disorder; CMT = childhood maltreatment

Table 2.

Cytokines	*MDD (n=22)*	*CMT (n=16)*	*Control (n=15)*	*P*	*Post Hoc*
IL-2	512.14 ± 109.12	417.86 ± 152.00	1060.90 ± 189.40	**< 0.02**	MDD=CMT<CTRL
IL-4	346.37 ± 87.48	162.88 ± 51.18	2997.29 ± 1710.04	**< 0.02**	MDD=CMT<CTRL
IL-6	3931.82 ± 880.15	3862.98 ± 1061.57	4867.81 ± 1532.65	0.16	NS
IL-10	1617.94 ± 413.02	1177.46 ± 659.64	2467.76 ± 956.16	0.05	NS
TNF-α	2034.02 ± 491.16	913.70 ± 456.02	2063.64 ± 593.13	**< 0.05**	CMT<MDD=CTRL
IFN-γ	2390.71 ± 548.54	1236.40 ± 500.92	2813.09 ± 767.76	0.12	NS

Figure 1


A
Control
MDD
CMT
Cortisol (nM)
15.0
12.5
10.0
7.5
5.0
2.5
0.0
***
##
08 AM
12 PM
08 PM


B
DHEAS (nM)
1.5
1.0
0.5
0.0
*
# #
Control
MDD
CMT

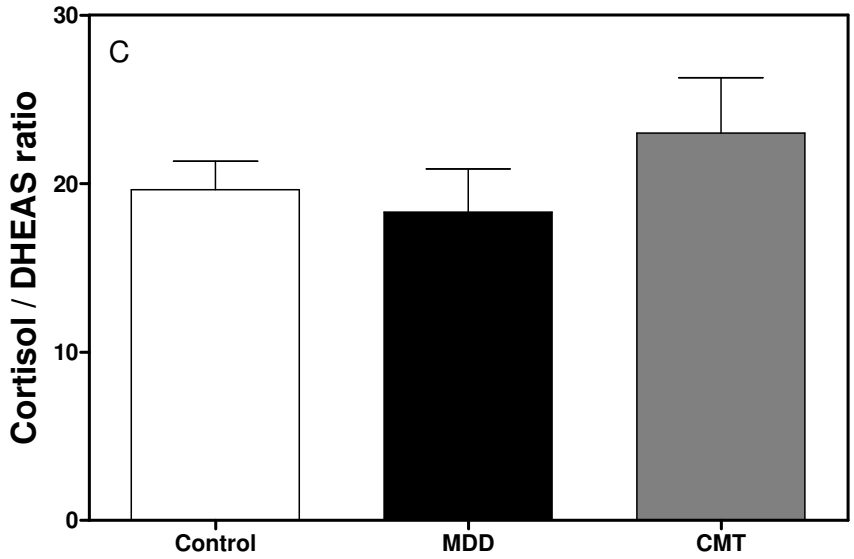

C
Cortisol / DHEAS ratio
30
20
10
0
Control
MDD
CMT

Figure 2

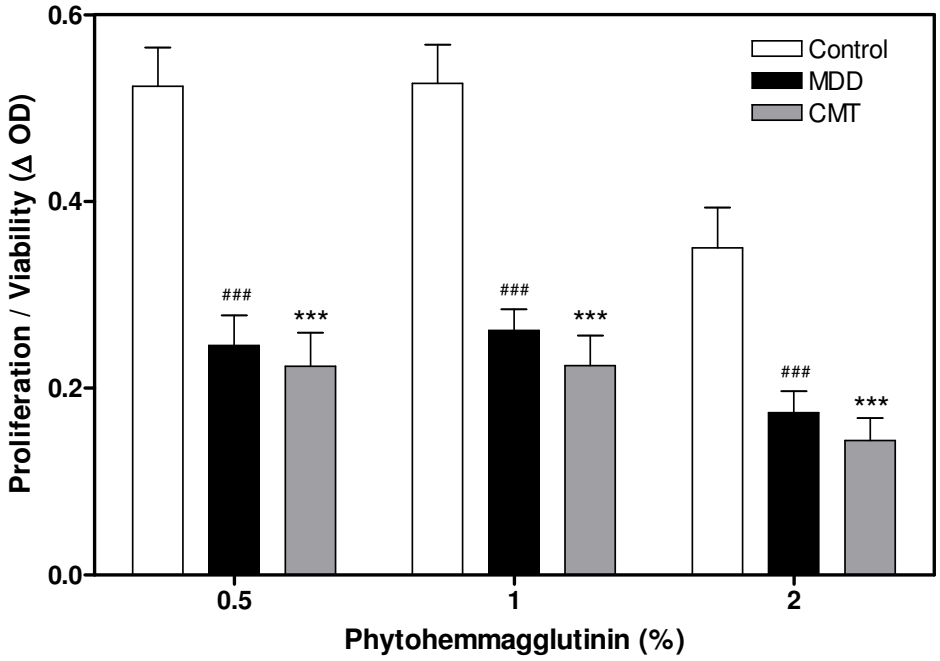
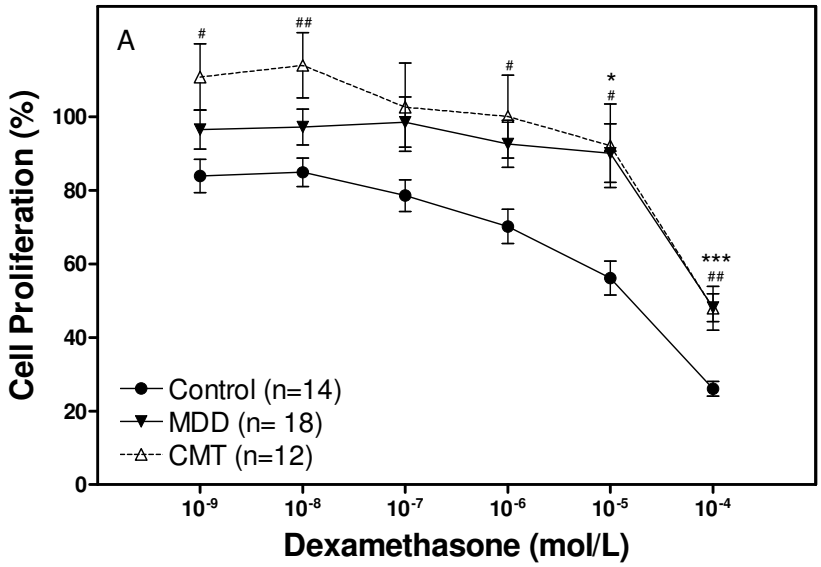
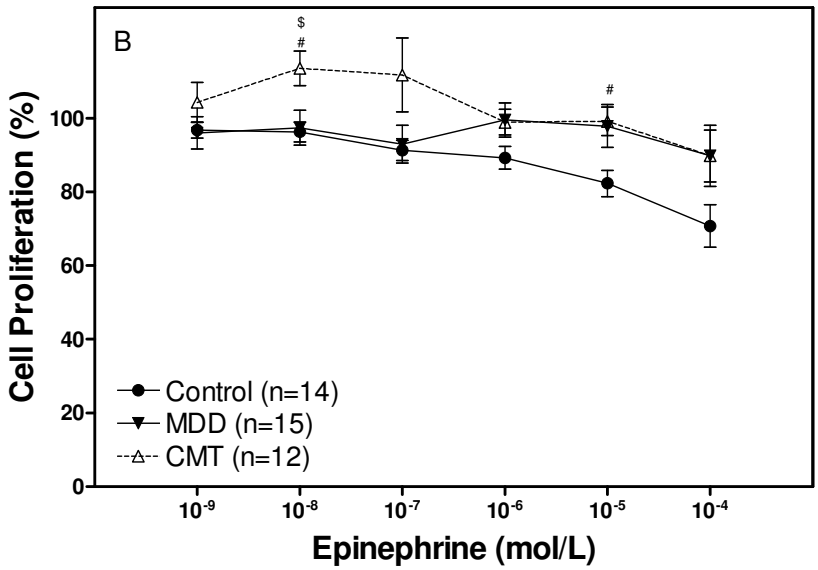

0.6
0.4
0.2
0.0
Proliferation / Viability (Δ OD)
Control
MDD
CMT
###
***
###
***
###
***
0.5
1
2
Phytohemmagglutinin (%)

Figure 3


A
Cell Proliferation (%)
Control (n=14)
MDD (n= 18)
CMT (n=12)
Dexamethasone (mol/L)


B
Cell Proliferation (%)
Control (n=14)
MDD (n=15)
CMT (n=12)
Epinephrine (mol/L)

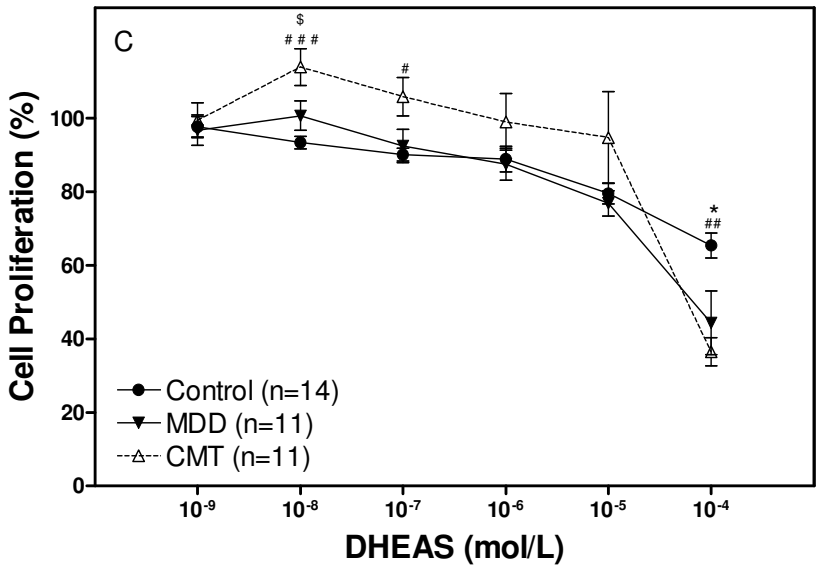

C
Cell Proliferation (%)
Control (n=14)
MDD (n=11)
CMT (n=11)
DHEAS (mol/L)

TABLE LEGENDS

Table 1. Demographic and psychosocial characteristics of subjects. BDI, Beck Depression inventory; BMI, Body Mass Index; CTQ, Childhood Trauma Questionnaire; PCL-C, Post-Traumatic Stress Disorder Checklist – Civilian Version. MDD, major depressive disorder. CMT, major depression with childhood maltreatment.

Table 2. Supernatant Th1/Th2 cytokine levels of stimulated PBMCs. Simultaneous measurement of six secreted cytokines from supernatant samples of PHA-stimulated cell cultures was performed by flow cytometry (Cytometric Bead Array). Statistical analysis was conducted using the Kruskal-Wallis one-way analysis of variance and checked with the Tukey's HSD post-hoc test. The significance level was set at $\alpha = 0.05$. MDD, major depressive disorder. CMT, major depression with childhood maltreatment.

FIGURE LEGENDS

Fig. 1. Salivary evaluation of adrenal secreted hormones. Cortisol and DHEAS levels were assessed on duplicates by radioimmunoassay (RIA). (**A**) Cortisol levels were measured across the day and (**B**) DHEAS levels were determined from a single morning salivary collection (08 a.m.). (**C**) Cortisol / DHEAS ratio was determined by calculating the relation between the morning (08 a.m.) salivary levels of both hormones. MDD, major depressive disorder. CMT, major depression with childhood maltreatment. Statistical significance differences are indicated: $^{\#\#}P < 0.01$ (CMT vs. Control) ; $^{*}P < 0.05$ and $^{***}P < 0.001$ (MDD vs. Control). Control (n=13), MDD (n=20), CMT (n=16).

Fig. 2. Assessment of T-cell proliferation. PBMCs were cultured with and without the T-cell selective mitogen phytohemagglutinin (PHA) for 96 h and proliferation estimated by colorimetric assays. Proliferation/viability was expressed as delta optical density (ΔOD = OD of stimulated – OD of non-stimulated cultures). MDD, major depressive disorder. CMT, major depression with childhood maltreatment. Statistical significance differences are indicated: $^{\#\#\#}P < 0.001$ (CMT vs. Control); $^{***}P < 0.001$ (MDD vs. Control). Control (n=14), MDD (n=21), CMT (n=15).

Fig. 3. Cellular sensitivity to adrenal hormones *in vitro*. Peripheral T-cell sensitivity to hormones was assessed by incubating PBMCs with 1% PHA and increasing concentrations of (**A**) dexamethasone (glucocorticoid agonist); (**B**) epinephrine and (**C**) DHEAS. Proliferation/viability was estimated by a colorimetric MTT assay. Data are shown as percentage of baseline cell proliferation (100% = PBMCs cultured with 1% PHA without hormones). MDD, major depressive disorder. CMT, major depression with childhood maltreatment. Statistical significance differences to isolated hormone concentrations are indicated: $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ (CMT vs. Control); $^{*}P < 0.05$ and $^{***}P < 0.001$ (MDD vs. Control); $^{\$}P < 0.05$ (CMT vs. MDD).

2.2 ARTIGO CIENTÍFICO

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Low Plasma Brain-Derived Neurotrophic Factor and Childhood Physical Neglect Are Associated with Verbal Memory Impairment in Major Depression - A Preliminary Report

Rodrigo Grassi-Oliveira [a], Lilian Milnitsky Stein [a], Rodrigo Pestana Lopes [b], Antonio L. Teixeira [d] and Moisés E. Bauer [b,c]

[a]Faculty of Psychology, PUCRS, Porto Alegre, Brazil;

[b]Institute of Biomedical Research, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre, Brazil;

[c]Faculty of Biosciences, PUCRS, Porto Alegre, Brazil;

[d]Department of Internal Medicine, School of Medicine, Federal University of Minas Gerais (UFMG), Belo Horizonte, Brazil;

Key words: Affective disorders, BDNF, childhood neglect, early life stress, major depression, memory.

Corresponding author: Dr. Moisés E. Bauer, Instituto de Pesquisas Biomédicas, Av. Ipiranga 6690, 2º andar. P.O. Box 1429. Porto Alegre, RS 90.610-000, Brazil. Email: mebauer@pucrs.br

ABSTRACT

Background: Early life stress has been suggested to mediate vulnerability to affective disorders. Animal models of repeated maternal separation have shown reduced brain-derived neurotrophic factor (BDNF) levels in specific brain regions implicated with hypothalamicpituitary-adrenal axis and memory formation. In addition, BDNF levels are also reduced in major depressive disorder (MDD) and bipolar disorder. The aim of this study was to investigate whether childhood physical neglect (CPN) and plasma BDNF levels would impact on memory performance in adult female subjects with recurrent major depression. **Methods:** Recurrent female MDD outpatients with CPN (MDD + CPN, $n = 17$) and without CPN (MDD, $n = 17$) and healthy control subjects ($n = 15$) were assessed for plasma BDNF content and verbal memory performance. Memory was assessed through the logical memory component of the Weschler Memory Scale-Revised for immediate and delayed recall. Brain-derived neurotrophic factor was assessed with enzyme-linked immunosorbent assays (ELISAs). **Results:** Major depressive disorder patients showed lower plasma BDNF concentrations than healthy control subjects ($p < .001$). Major depressive disorder + CPN had even lower BDNF levels compared with control subjects and MDD ($p < .05$). Brain-derived neurotrophic factor levels were negatively related to psychological morbidity and positively correlated to memory performance. Regression models showed that severity of self-reported CPN and low plasma BDNF predicted impairment on immediate verbal recall. Delayed recall impairment was predicted by severity of CPN and depression and memory retention by posttraumatic stress disorder (PTSD) severity symptoms. **Conclusion:** Our data suggest that CPN and plasma BDNF are important factors associated with depression and verbal memory performance, particularly with encoding processes.

Introduction

Early life stress has been suggested to mediate vulnerability to affective disorders including unipolar depression (Heim and Nemeroff, 2001). A variety of studies has shown the close relationship between childhood abuse (particularly sexual and physical abuse) and neurobiological consequences in adult life (Teicher et al., 2006). On the other hand, there are few studies that have examined the psychobiological consequences of neglect forms of childhood maltreatment (Widom et al., 2007).

Maternal separation (an animal model for early life stress in which rat pups are deprived of maternal contact once or repeatedly during the first postnatal weeks) could program widespread and lifelong changes in various transmitter systems that regulate the hypothalamus-pituitary-adrenal (HPA) axis (Champagne and Meaney, 2001). Some authors have suggested that repeated maternal separation could reduce brain-derived neurotrophic factor (BDNF) levels in specific brain regions (i.e., hippocampus) implicated with HPA axis and memory formation early in development (Duman, 2002). Despite any direct evidence implicated with maternal deprivation, BDNF activity, and memory performance, a reduction in BDNF activity in hippocampus of rats has been associated with a marked deficit in memory persistence (Bekinschtein et al., 2007). Brain-derived neurotrophic factor levels have been found to be reduced in major depression or bipolar disorder (Machado-Vieira et al., 2007). However, even considering that in patients with mood disorders BDNF levels are lower than among those with a history of trauma (Kauer-Sant'Anna et al., 2007), it is largely unknown to what extent BDNF levels are related to childhood physical neglect (CPN) and memory performance in major depression. It has been shown that specifically verbal declarative memory and small hippocampal volume could be related to childhood sexual abuse (Bremner et al., 2003b). Thus, the aim of this study was to investigate

whether CPN and plasma BDNF levels would impact on verbal memory performance in adult female subjects with major depressive disorder (MDD).

Materials and Methods

Subjects and Clinical Assessment

Sixty MDD outpatients (all female subjects, 20 – 55 years) from an affective unit at the Hospital Presidente Vargas (Porto Alegre, Brazil) were interviewed by two well-trained clinical psychiatrists with the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) to confirm the diagnosis of recurrent MDD. A group of 17 outpatients with recurrent major depression without CPN (MDD) and 17 outpatients with recurrent major depression with CPN (MDD + CPN) were enrolled in this study. All patients had taken a stable dosage of antidepressant for at least 3 months prior to the experiment. Severity of depressive symptoms was evaluated by Beck Depression Inventory (BDI) (Gorenstein and Andrade, 1996) and posttraumatic stress disorder (PTSD) symptoms by PTSD Checklist-Civilian Version (PCL-C) (Lang et al., 2003). Childhood physical neglect was assessed through the Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 2003, Grassi-Oliveira et al., 2006b) and was classified from moderate to extreme according to the cutoff point postulated by Bernstein *et al.* (Bernstein et al., 2003) (percentile 90) for adult female subjects. The therapists' ratings were used as a stringent test of validity of retrospective reports of childhood neglect, showing a good criterion-related validity ($r = .45$, $p < .001$), whereas convergent and discriminant validity were demonstrated with a structured trauma interview (Bernstein et al., 2003). Participants presenting past or current Axis I disorders other than MDD, severe or unstable clinical illness, neurological disorder, psychotic symptoms, or any psychoactive substance use in last 30 days (except nicotine, caffeine, and antidepressants) were excluded.

Ninety-four healthy hospital employees (all female subjects, 20–50 years) were screened by psychiatric disorders with SCID-I, BDI, PCL-C, and CTQ. All participants with any past or current Axis I disorder, any history of childhood maltreatment, severe or unstable clinical illness, neurological disorder, or any psychoactive substance use in the last 30 days (except nicotine and caffeine) were excluded. The remaining participants consisted of the control group (n = 15). The current research was approved by Pontifical Catholic University of Rio Grande do Sul (PUCRS) Ethics Committee. Written informed consent was obtained from all participants.

Memory Assessment

Memory tests were performed between 8:30 AM and 9:30 AM, 30 minutes after blood was drawn. The Logical Memory (LM) test was administered to all subjects. Logical Memory is a verbal declarative memory subtest from the Wechsler Memory Scale- Revised (WMS-R) (Wechsler, 1987), and it is a well-defined verbal memory test that has been used extensively in the literature (Savitz et al., 2007). The LM was administered and scored by two well-trained psychologists according to the manual guidelines. The test generates a score for immediate verbal recall (IVR) and 30-minute delayed verbal recall (DVR). The percent of memory retention (%R) represents the IVR divided by DVR.

Plasma Brain-Derived Neurotrophic Factor Levels

All participants were instructed not to eat or take medication for at least 8 hours before the blood draw. Human blood was collected from 8:00 AM to 9:00 AM, before memory assessments. Plasma was separated within 30 minutes and the supernatant was stored at -80°C for up to 6 months. For BDNF measurement, a commercially available BDNF immunoassay enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis,

Minnesota) was used, following manufacturer's procedures. All samples were assayed on duplicates. The detection limits for these assays were 20pg/mL. Brain-derived neurotrophic factor levels are expressed as pg/mL.

Statistical Analysis

All variables were tested for normality of distribution by means of the Kolmogorov-Smirnov test. Group mean differences were assessed by means of the analysis of variance (ANOVA). Multiple comparisons among group mean differences were checked with the Tukey's honestly significant difference (HSD) test. The relationships between demographic/psychosocial variables and memory performance were explored by Pearson's correlation. The distribution of BDNF concentrations failed in normality test and data were thus log transformed. Multiple regression analyses were employed to control for potential confounding variables and determine the independent correlations between memory performance, childhood neglect, and plasma BDNF. Factors that showed significant association with each memory dependent variable were included in the correspondent regression equation following the stepwise method. A *p*-value of less than .20 was required for a factor to be included and retained in the analysis. Due to the high variability on BDNF plasma concentrations found in the literature (from 22 pg/mL to 14,000 pg/mL) (Karege et al., 2005, Lee et al., 2007), we performed a dummy coding in which subjects with concentrations 2 SD below the mean of the control subjects (2347.03 pg/mL) were considered with low plasma BDNF (BDNF $\leq$ 2347.03 = 1; BDNF > 2347.03 = 0). The significance level was set at $\alpha = .05$ (two-tailed). Statistical analyses were performed with the SPSS 15.0 for Windows (SPSS Inc., Chicago, Illinois). Results are expressed as mean ± SD in all figures and tables.

Results

The demographic, psychosocial and clinical characteristics of samples are shown in Table 1. All groups were very similar regarding age, education, and social status, and both depression groups were homogeneous in terms of severity of depression and PTSD symptoms, illness duration, time of treatment, and type of antidepressant treatment. The MDD + CPN group showed impairment in immediate ($p < .05$) and delayed recall ($p < .01$) in comparison with other groups, except for memory retention. Figure 1 shows that MDD groups presented significantly lower BDNF plasma concentrations than the healthy control group ($p < .001$). In addition, MDD patients with CPN had even lower BDNF levels when compared with patients without CPN ($p < .05$). This difference seems to be true even after adjusting for the type of antidepressant used, $F(2,31) = 3.51$, $p < .05$.

Zero-order analyses showed significant correlations of IVR with years of education ($r = .39$, $p = .006$), severity of depression (BDI) ($r = - .49$, $p < .001$), PTSD symptoms (PCL-C) ($r = - .38$, $p = .006$), CPN (CTQ) ($r = - .54$, $p < .001$), and plasma BDNF (log) ($r = .43$, $p = .002$). Moreover, DVR was associated with the same variables: years of education ($r = .34$, $p = .016$), depression severity ($r = - .52$, $p < .001$), PTSD symptoms ($r = - .43$, $p = .002$), CPN ($r = -.53$, $p < .001$), and plasma BDNF ($r = .40$, $p = .004$). On the other hand, the %R was only correlated with severity of depression ($r = - .29$, $p = .038$) and PTSD symptoms ($r = - .30$, $p = .035$).

Multivariate analyses were employed to assess the independent roles of CPN and plasma BDNF on memory performance. Thus IVR, DVR, and %R were entered as dependent variables in these regression analyses. Independent variables entered into each multivariate model were plasma BDNF, low plasma BDNF, and CPN. Furthermore, the multivariate analyses were ad-

justed by entering in the equation those variables previously correlated with their dependent variables (memory performance). The model established that the factors correlated with memory measures also included age and MDD diagnosis (MDD = 1, non-MDD = 0). During the regression models, there was no evidence of high colinearity between selected variables, and all variance inflation factor (VIF) values were <2. For IVR stepwise regression, the variables selected ($F \leq .05$) were CPN and low BDNF [$R = .62$, adj $R^2 = .36$; $F(2,46) = 14.60$, $p < .001$]. For DVR, the variables selected were CPN and severity of depression [$R = .62$, adj $R^2 = .35$; $F(2,46) = 14.37$, $p = .008$]. For %R regression equation, the only variable entered was PTSD severity [$R = .30$, adj $R^2 = .07$; $F(2,46) = 4.71$, $p = .035$].

When plasma BDNF concentrations were entered in the equation, the selected variables predicting IVR were CPN severity, depression severity, and years of education [$R = .66$, adj $R^2 = .40$; $F(3,45) = 11.65$, $p < .001$]. No linear correlation was found between plasma BDNF and immediate recall ($r = - .03$, $p = .83$). However, when low plasma BDNF was included in the regression, severity of CPN and low plasma BDNF were found to be predictors of immediate recall impairment after adjusting for potential confoundable variables. On the other hand, low plasma BDNF was not a predictor of delayed recall deficits, in contrast to CPN and depression severity. Decrease in memory retention was only predicted by PTSD symptoms.

Discussion

To our knowledge, this is the first study addressing the role of BDNF levels on cognitive performance in MDD patients with a history of CPN. Patients with MDD showed low plasma BDNF concentrations in accordance with previous work (Aydemir et al., 2006, Shimizu et al., 2003). Interestingly, patients with a history of CPN had even lower BDNF levels than patients

without CPN, which is consistent with previous data from low serum BDNF in bipolar patients with trauma history. Brain-derived neurotrophic factor levels were negatively related to psychological morbidity and positively correlated with memory performance. Multivariate regression models showed that severity of self-reported CPN and low plasma BDNF predicted impairment on immediate verbal recall. Delayed recall performance was predicted by severity of CPN and depression, and memory retention was predicted by severity of PTSD symptoms. Although BDNF valine (Val)66methionine (Met) polymorphism has been implicated in verbal memory deficits (Harris et al., 2006), the direct involvement of peripheral BDNF on verbal memory is still largely unknown. To our knowledge, there are few preliminary analyses indicating that BDNF serum concentration reflects some aspect of neuronal plasticity, as indicated by its association with in vivo level of cerebral N-acetylaspartate, a well-established marker of neuronal integrity (Lang et al., 2007). Our data further support the role of BDNF on memory performance.

Multivariate analyses were employed in this study to better understand the independent roles of CPN and BDNF levels on memory performance. Our analyses revealed that exposure to CPN was related to degree of immediate and delayed verbal memory impairment, corroborating previous studies that investigated this relationship with childhood exposure to sexual abuse (Navalta et al., 2006, Stein et al., 1999). In addition, low plasma BDNF was associated with lower scores on the WMS-R logical component for immediate but not delayed recall, which is consistent with a genotyping study (Ho et al., 2006, Haaland et al., 2003). These associations were found after adjusting for age, years of education, PTSD symptoms, depression severity, and severity of MDD. However, despite depression severity being negatively associated with DVR performance, low BDNF levels were not. Thus, depression severity predicted delayed verbal memory impairment independent of BDNF activity. Furthermore, the only predictor of verbal

memory retention impairment was PTSD symptoms severity, in accordance with previous studies (Bremner et al., 2003b).

Our data also indicated that CPN and plasma BDNF may be important factors associated with encoding processes of memory performance. The LM component of WMS-R reflects short-term verbal memory. It is assumed that IVR reflects encoding and retrieval of information previously studied and DVR reflects initial encoding indirectly and storage and retrieval of the information that was initially encoded (Haaland et al., 2003). Considering that IVR is related to acquisition of information (Brooks et al., 2006), it is hypothesized that low plasma BDNF effects on immediate recall reflect deterioration in encoding and retrieval more than storage processes. On the other hand, CPN seems to be related to general impairment in short-term verbal memory, since it was negatively associated with IVR and DVR scores. Since %R is a delayed recall measure adjusted for immediate recall performance and no effect of CPN was related to memory retention, this general impairment could be associated predominantly with short-term verbal memory encoding processes.

Adverse prenatal environment and environmental enrichment are able to produce structural defects in brain areas associated with memory formation, including the hippocampus. In that sense, a study with a cohort of 1116 5-year-old twin pairs showed that maternal warmth, stimulating activities, and children's outgoing temperament appeared to promote positive adjustment in children exposed to socioeconomic deprivation (Kim-Cohen, Moffitt et al. 4004). Interestingly, offspring of rat mothers with high levels of pup licking and grooming and arched-back nursing had increased hippocampal cholinergic innervation, memory, and spatial learning and an increased expression of BDNF messenger RNA (mRNA) (Liu et al., 2000), as well as maternal aggression and maternal separation being associated with low neurotrophin activity in adult rats

(Alleva and Santucci, 2001). Together, these observations could raise some ideas about early life stress and vulnerability to major depression (Penza, Heim et al. 2003) and lead us to the hypothesis by Post (Post, 1992) about the transduction of psychosocial stress into mood episodes, where both sensitization to stressors and episode sensitization occur in major affective disorders and become encoded at the level of gene expression.

There are some limitations in this study to be discussed. First, sample sizes were rather small in this study. The statistical power is relatively low to moderate and we cannot exclude the possibility of a type 2 error. It should be noted, however, that it was very difficult to recruit healthy control subjects with no past or current psychopathology and any kind of childhood maltreatment from the same low socioeconomic status. The stringent inclusion/exclusion criteria limited the sample size but yielded strictly healthy control subjects. Replication with large samples and longitudinal follow-up will be needed to overcome these limitations. Second, our interpretations are based on findings of a single memory test and further studies should preferably include several memory tests. Third, recurrent MDD participants were using antidepressants. Despite there being some evidence that antidepressant treatment significantly increases serum BDNF in depressive women with low serum BDNF (Huang et al., 2008), we cannot discard the medication effects on plasma BDNF in this study. It should be noted, however, that our groups of patients were homogenously treated with common antidepressants. Furthermore, it is extremely difficult to investigate unmedicated patients with recurrent MDD. Fourth, although BDNF from the central nervous system may contribute to the interindividual variance of BDNF levels in plasma (Lommatzch, Zingler et al. 2005), plasma BDNF function is poorly understood (Karege, Bondolfi et al. 2005). Thus, the specific meaning of low plasma BDNF to the complex molecular mechanisms of depression is still unknown. Fifth, another limitation is the possibility of memory

bias that could be associated with retrospective rating of CPN and severity of depression. Sixth, changes in menstrual cycle could have potentially yielded changes in BDNF levels. Although women in the second half of the menstrual cycle showed higher platelet levels of BDNF than women in the first half of the menstrual cycle or postmenopausal women, plasma BDNF levels were not affected (Lommatzch, Zingler et al. 2005). This is probably because plasma BDNF is not dependent on platelet activity (Karege, Bondolfi et al. 2005). In addition, there is no difference in cognitive performance between phases of the menstrual cycle (Gordon and Lee, 1993). Finally, it would be interesting to provide data on other neurotrophins, such as neuron growth factor (NGF), insulin-like growth factor (IGF)-1, or neurotrophin-3, to discuss the specificity of our findings in MDD patients.

Taking into consideration these limitations, our data further support the role of childhood physical neglect and plasma BDNF levels on major depression and verbal memory performance, particularly involving encoding processes. More studies are necessary to replicate these findings in a powered design to clarify the complex relationships between childhood neglect, BDNF, and verbal memory functioning. Further studies should consider investigating the relationships between cognitive impairment, early stress sensitization, and allostatic load in MDD, following the new perspectives about pathophysiology of major affective disorder (Kapczinski et al., 2008). Additional studies will also be needed to determine if decreased BDNF reflects a state or trait marker and to determine the functional significance of altered peripheral BDNF.

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Table 1.

	MDD[a] + CPN (n = 17)		MDD[a] (n = 17)		Control Subjects (n=15)			
	M	SD ±	M	SD ±	M	SD ±	F/χ^2	*p*
Age (years)	39.35	9.32	39.53	7.96	36.47	6.31	.71	.49
Education (years)	9.47	4.51	7.65	2.93	9.00	2.50	1.26	.29
Income (US$/month)	564.93	316.99	706.56	389.33	620.66	182.61	.82	.44
PTSD[b]	58.70[e]	11.65	52.82[e]	15.93	22.80[f]	5.22	40.32	< .001
DEPRESSION[c]	30.82[e]	10.12	26.35[e]	11.63	4.53[f]	3.90	35.42	< .001
CTQ - CPN	14.17[e]	4.17	6.64[e]	1.53	7.2[f]	1.65	77.21	< .001
Illness Duration (years)	10.64	4.56	11.88	6.50	—	—	.41	.52
Treatment Time (years)	7.76	3.28	6.58	3.26	—	—	1.09	.30
Antidepressant							1.25	.53
Tricyclic	52.9%		41.2%		—	—	.49	.73
SSRI	41.2%		41.2%		—	—	.00	1.0
Other	5.9%		17.6%		—	—	1.13	.28
IVR[d]	15.76[e,f]	6.63	21.47	7.99	22.87[f]	6.30	4.68	.014
DVR[d]	11.18[e,f]	5.50	17.06	7.98	19.0[f]	7.30	5.53	.007
Retention	69.19	18.07	81.04	26.16	81.52	18.61	1.77	.18

Figure 1.

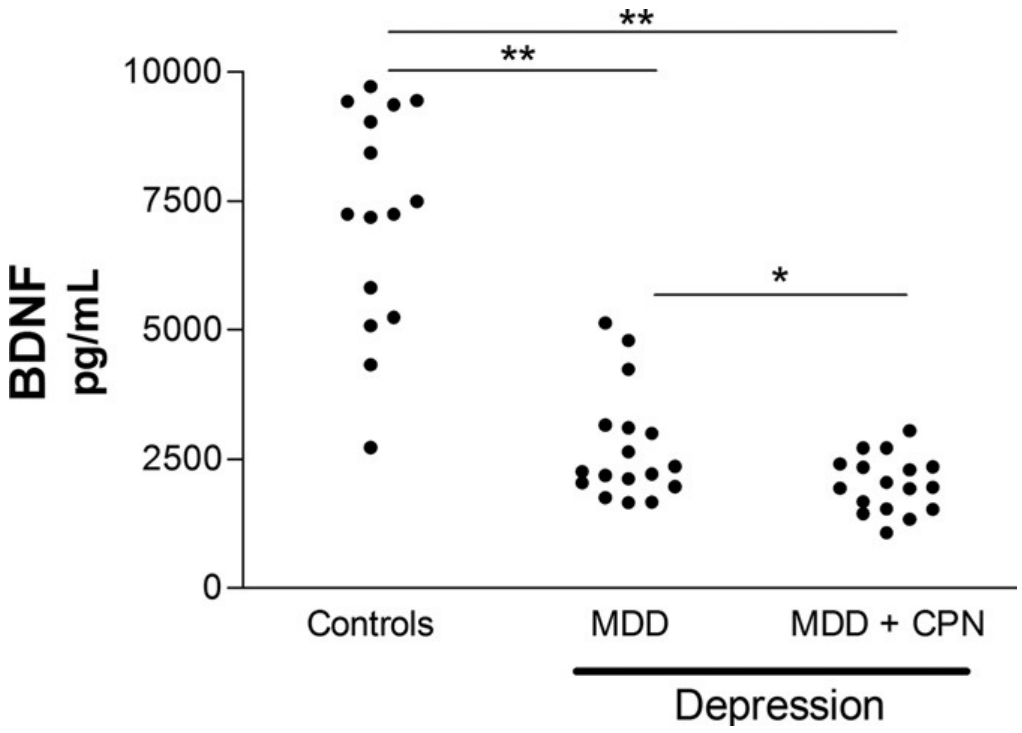
**
**
*
10000
7500
5000
2500
0
BDNF
pg/mL
Controls
MDD
MDD + CPN
Depression

TABLE LEGENDS

Table 1. Demographic and psychosocial characteristics of samples.

Note: All *df* = 2,46. Memory retention was calculated by R% = (DVR/IVR) x 100.

BDI, Beck Depression Inventory; CPN, childhood physical neglect; CTQ, Childhood Trauma Questionnaire; DVR, delayed verbal recall; HSD, honestly significant difference; IVR, immediate verbal recall; LM/WMS-R, Logical Memory/Wechsler Memory Scale-Revised; MDD, major depressive disorder, recurrent; PCL-C, PTSD Checklist-Civilian Version; PTSD, posttraumatic stress disorder; SCID-I, Structured Clinical Interview for DSM-IV Axis I Disorders; SSRI, selective serotonin reuptake inhibitors.

[a]SCID-I; [b]PCL-C; [c]BDI; [d]LM/WMS-R.

Statistical significance differences are indicated:

[e]$p < .05$ versus control subjects.

[f]$p < .05$ versus MDD (Tukey HSD).

FIGURE LEGENDS

Figure 1. Plasma BDNF levels between patients with major depression and healthy control subjects. Statistical significant differences are based on log transformation of BDNF levels: *$p < .05$, **$p < .001$. BDNF, brain-derived neurotrophic factor; controls, healthy participants; CPN, childhood physical neglect; MDD, major depressive disorder.

2.3 ARTIGO CIENTÍFICO

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Increased soluble tumor necrosis factor-α receptors in patients with major depressive disorder

Rodrigo Grassi-Oliveira [1], Elisa Brietzke [2], Júlio C. Pezzi [2], Rodrigo Pestana Lopes [3], Antonio L. Teixeira [4] and Moisés E. Bauer [3]

[1]Postgraduate Program Psychology, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil;

[2]Psychiatry Department of Federal University of Rio Grande do Sul, Porto Alegre, Brazil;

[3]Institute of Biomedical Research, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil;

[4]Department of Internal Medicine, School of Medicine, Federal University of Minas Gerais, Belo Horizonte, Brazil.

Key words: Biological markers, child abuse, cytokines, mood disorder, inflammation.

Corresponding author: Rodrigo Grassi-Oliveira, Av. Ipiranga 6681, Prédio 11, sala 933. Porto Alegre, RS 90.619-900, Brazil. Email: rodrigo_grassi@terra.com.br

ABSTRACT

Aim: Several lines of evidence suggest that major depressive disorder is associated with an inflammatory status. Tumor necrosis factor-α has been investigated as a potential molecular target in mood disorders. Tumor necrosis factor-α exerts its activity through binding to specific cell membrane receptors named as TNFR1 and TNFR2. The aim of the present study was to investigate soluble plasma TNFR1 (sTNFR1) and TNFR2 levels (sTNFR2) in major depressive disorder patients.

Methods: Female outpatients with major depressive disorder (n = 30) were compared with a healthy control group (n = 19). Severity of depressive symptoms was evaluated on Beck Depression Inventory; post-traumatic stress disorder (PTSD) symptoms were evaluated on PTSD Checklist–Civilian Version; and childhood abuse and neglect on the Childhood Trauma Questionnaire. Plasma tumor necrosis factor-a and its soluble receptors were measured by ELISA.

Results: Patients had no changes in tumor necrosis factor-a concentrations but did have increased sTNFR1 (P < 0.001) and sTNFR2 (P < 0.001) levels compared to controls. Plasma level of sTNFR1 was positively predicted by age (B = 0.25, P = 0.05) and PTSD-like symptoms (B = 0.41, P = 0.002) and plasma levels of sTNFR2 by depression severity (B = 0.67, P < 0.001).

Conclusion: Soluble tumor necrosis factor-α receptors could be reliable markers of inflammatory activity in major depression.

Introduction

Recent studies report that patients with major depressive disorder (MDD) show activated inflammatory status, with increased proinflammatory cytokines, acute-phase proteins, and increased expression of chemokines and adhesion molecules (Raison et al., 2006, Dome et al., 2008, Yang et al., 2007). Tumor necrosis factor-a (TNF-a) has been investigated as a potential molecular target in mood disorders (Brietzke and Kapczinski, 2008), and clinical improvement was associated with decreased serum levels (Himmerich et al., 2006, Lanquillon et al., 2000). In addition, specific depressive symptoms (e.g. suicidal behavior) were associated with increased cytokine levels (Kim et al., 2008).

Tumor necrosis factor-a exerts its main effects by binding two specific receptors, TNFR1 (55 kDa) and TNFR2 (75 kDa) (Vandenabeele et al., 1995). The soluble forms of the TNF-a receptors (sTNFR1 and sTNFR2), which represent the extracellular portions of membrane-associated TNF-a receptors, play a role as modulators of the biological TNF-a activity (Diez-Ruiz et al., 1995). The binding of TNF-a to TNFR1 leads to recruitment of associated death domain protein mediated apoptosis and nuclear factor-kappa B (NF-kB) activation (Chen and Goeddel, 2002). TNFR2 is associated only with NF-kB activation and seems to have a dominant role in suppressing TNF-mediated inflammatory responses (Carpentier et al., 2004). Specifically, soluble TNF-a receptors seem to prolong the half-life of TNF-a by protecting this cytokine against proteolytic degradation and ultimately influencing neuroprotective or neurotoxic effects (Granell et al., 2004, Pouly et al., 2000).

In humans, measurement of circulating levels of the two soluble receptors is useful to determine the overall production of TNF-a (Alaaeddine et al., 1997). Because TNF-a may

be less stable than its soluble receptors, some authors suggest that sTNFR1 and sTNFR2 are more reliable markers of TNF-a activity and, as consequence, of inflammatory activity (Kronfol and Remick, 2000). There are only a few recent studies indirectly addressing an increase in sTNFR1 levels in depressive disorder (Friebe et al., 2007, Moorman et al., 2007) and none addressing sTNFR2. In addition, post-traumatic stress disorder (PTSD) symptoms and early life stress are associated with a low-grade systemic pro-inflammatory state, enhancing TNF-a activity (Veenema et al., 2008, von Kanel et al., 2007). Specifically childhood maltreatment has been associated with adult inflammatory status (Danese et al., 2008, Danese et al., 2007). The aim of the present study was to evaluate sTNFR1, sTNFR2 and TNF-a plasma levels in MDD female outpatients. A secondary goal of this study was to assess possible influences of PTSD symptoms and history of childhood maltreatment with sTNFR1 and sTNFR2 plasma levels.

Methods

Subjects and clinical assessment

Thirty MDD female outpatients in partial remission (20–55 years of age) were recruited from of an affective disorder program at the Hospital Presidente Vargas (Porto Alegre, Brazil). Diagnosis of MDD was confirmed using the Structural Clinical Interview for DSM-IV Axis I Disorders (SCID-I). Social status was evaluated on Hollingshead's Index of Social Status (Cirino et al., 2002). Severity of depressive symptoms was evaluated on Beck Depression Inventory (BDI) (Beck et al., 1961, Gorenstein and Andrade, 1996), post-traumatic stress disorder (PTSD)-like symptoms, on the PTSD Checklist–Civilian Version (PCL-C) (Berger et al., 2004b, Lang et al., 2003), and childhood abuse and neglect, on

Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 2003, Grassi-Oliveira et al., 2006b). All patients had been on a stable dose of antidepressant monotherapy for at least 3 months and were free of other psychiatric and non-psychiatric drug use for 4 weeks before blood collection. Exclusion criteria were: Axis I comorbidities, severe or unstable clinical illness or illness associated with abnormal immunological parameters, neurological disorder, psychotic symptoms, or any psychoactive substance used in the last 30 days (excepting nicotine, caffeine and antidepressants).

Ninety-four healthy hospital employees (all female, 20–50 years of age) from low social status were evaluated on SCID-I, Hollingshead's Index, BDI, PCL-C and CTQ. All participants with past or current Axis I disorder, severe or unstable clinical illness or illness associated with abnormal immunological parameters, neurological disorder, moderate to severe childhood abuse and neglect or psychoactive substance use in the last 30 days (excepting nicotine and caffeine) were excluded. The remaining participants comprised the control group (n = 19). The present study was approved by Pontifical Catholic University of Rio Grande do Sul (PUCRS) Ethics Committee. Written informed consent was obtained from all participants.

Plasma sTNFR1 and sTNFR2

Whole blood was collected between 08.00 and 09.00 hours and participants had been instructed not to eat or take medication for at least 8 h before blood collection. Plasma was separated within 30 min and the supernatant was stored at -80°C for up to 6 months. Plasma TNF-a, sTNFR1 and sTNFR2 were measured according to the procedures supplied by the manufacturer and using ELISA kits for sTNFR1 and sTNFR2 (DuoSet, R&D Systems,

Minneapolis, MN, USA) as routinely performed at our lab (Alessandri et al., 2006). All samples were assayed on duplicate. The detection limits were 25 pg/mL for TNF-a, and 12 pg/mL for both soluble receptors. Values below the detection limits were assumed to be zero. Concentration is expressed as pg/mL.

Statistical analysis

All variables were tested for normality of distribution by means of the Kolmogorov–Smirnov test. Student's *t*-test was performed to compare demographic and clinical characteristics of groups. Mann–Whitney *U*-test was used to assess group differences in variables that failed the normality tests. Exploratory correlation analyses between sTNFR1 and sTNFR2 levels and demographic and clinical parameters were performed using Spearman's correlation coefficient. Analysis of covariance (ANCOVA) was used to compare group mean differences using factors that had significant association with each soluble TNF-a receptors. Multiple regression analysis was further performed to assess the independent weight of variables that could affect sTNFR1 and sTNFR2 concentration. The significance level was set at $\alpha = 0.05$ (two-tailed). Statistical analyses were performed using SPSS 15.0 (SPSS, Chicago, IL, USA). All values are presented as mean ± SD.

Results

The demographic and clinical characteristics of cohorts are shown in Table 1. Both groups were homogeneous regarding age, body mass index, education and social status. Patients with MDD reported 11.5 ± 5.58 years of illness duration and 7.56 ± 3.19 years of formal treatment 46.7% of patients were using tricyclic antidepressants ($n = 14$), 43.3% selective serotonin re-uptake inhibitors ($n = 13$), and 10% was currently using other antide-

pressants (n = 3). The type of antidepressant did not affect sTNFR1 (F = 1.74, P = n.s.) or sTNFR2 (F = 1.03, P = n.s.) plasma levels.

Zero-order analyses indicated a significant correlation between sTNFR1 and sTNFR2 (r = 0.71, $P < 0.001$). Moreover, sTNFR1 was found to be associated with age (r = 0.36, P = 0.001), severity of depression (BDI; r = 0.47, P = 0.001) and PTSD-like symptoms (PCL-C; r = 0.48, $P < 0.001$). In addition, sTNFR2 was correlated with severity of depression (r = 0.67, $P < 0.001$), PTSD-like symptoms (r = 0.62, $P < 0.001$), and childhood trauma severity (CTQ; r = 0.37, P = 0.008). The TNF-a plasma levels were similarly low or under the detection limit in both the control and MDD group (U = 239.5, P = n.s.; Fig. 1).

In contrast, analyses of covariance were used to assess mean group differences controlling for all variables correlated with soluble TNF-a receptors. Plasma sTNFR1 and sTNFR2 were detectable in all control and MDD subjects. After adjusting for previously correlated variables, patients with recurrent MDD had significantly higher concentration of sTNFR1 (447.71 ± 118.96 pg/mL) in comparison to healthy controls (298.62 ± 95.99 pg/mL) (F = 6.13, $P < 0.001$; Fig. 2a). Similarly, concentration of sTNFR2 was greater in MDD patients (2223.98 ± 303.56 pg/mL) than in control subjects (1627.15 ± 272 pg/mL; F = 10.8, $P < 0.001$; Fig. 2b).

Considering the results obtained on exploratory analyses, we built a theoretical model in which plasma sTNFR2 level was predicted by depression severity (BDI), PTSD-like symptoms (PCL-C) and by childhood trauma severity (CTQ). In contrast, the variability of sTNFR1 concentration was investigated with regard to age (years), depression severity and PTSD-like symptoms. Thus, multiple linear regression with the stepwise method was used

to assess the independent role of each variable on sTNFR2 and sTNFR1 plasma levels. In regression models there was no evidence of high colinearity between selected variables, and all variance inflation factors (VIF) were <1. Using sTNFR2 as a dependent variable, the only selected variable ($F \leq 0.05$) to be included in the equation was depression severity (B = 0.67, $P < 0.001$; $R = 0.67$, adjusted $R2 = 0.44$; $F(1,47) = 39.26$, $P < 0.001$). This variable was thus considered to be an important predictor of sTNFR2 concentration after adjusting the slope of the line for severity of PTSD-like symptoms and childhood maltreatment. In addition, level of sTNFR1 was independently predicted by PTSD-like symptoms (B = 0.41, $P = 0.002$) and age (B = 0.25, $P = 0.05$) after adjusting for depression severity (R = 0.54, adjusted R2 = 0.26; $F(2,46) = 9.51$, $P < 0.001$).

Discussion

To our knowledge this is the first study that objectively assessed plasma soluble TNF-a receptors in recurrent MDD. The sTNFR1 and sTNFR2 plasma levels were significantly increased in MDD, despite unchanged TNF-a concentrations. Multivariate analysis indicated that sTNFR2 levels are influenced by depression severity. Moreover, levels of sTNFR1 were independently predicted by PTSD-like symptoms and age.

Tumor necrosis factor-a receptors seem to have a potential antagonistic function with respect to neuronal survival upon exogenous stress signals and/or tissue damage (Fontaine et al., 2002). There is some evidence suggesting that TNFR2 is involved with TNF-mediated antiapoptotic pathways (Marchetti et al., 2004) given the TNF-mediated apoptotic pathway via TNFR1 (Wajant et al., 2003). Therefore TNFR2 is related to neuroprotection and TNFR1 to neurotoxicity. Although the intracellular effects of TNFR2 are not com-

pletely known, TNFR1 depends largely on the nuclear translocation of the transcription NF-kB. The duration of NF-kB activation is critical to achieve significant tissue protection (Beg and Baltimore, 1996). Cellular toxicity was observed in experiments in which TNFR1 induced a rapid but transient NF-kB response and TNFR2 produced a more persistent response (Marchetti et al., 2004). In contrast, soluble forms of TNFa receptors are correlated with the modulation effect of TNF-a at the site of synthesis and the facilitation of its transport to distant organs, promoting its systemic effects (Granell et al., 2004). The hypothesis regarding the TNF-a neutralizing effect is not attractive because a 30–300-fold molar excess of TNF-a soluble receptors is required to inhibit the cytotoxic action of TNF-a (Van Zee et al., 1992). Thus it is suggested that the role of TNF-a soluble receptor is related to stabilizing the trimeric structure of TNF-a more than buffering the effects of this cytokine (Granell et al., 2004). In the present study the MDD patients had increased TNF-a soluble receptors despite similar TNF-a plasma levels compared with controls, indicating an increased TNF-a activity. Therefore it is possible that both pro-apoptotic and anti-apoptotic pathways could be enhanced. The activation of the same intracellular key molecule (NFkB) could trigger a completely different signal output (neuroprotection vs neurotoxicity) due to a differential duration of the active state of this molecule.

Neurodegeneration, reduced neuroprotection and neuronal repair are common pathological features of major depression (Leonard, 2007). The sTNFR1 and sTNFR2 could be considered as potential biological markers associated with such features, despite the fact that studies are needed to confirm such speculation. In contrast, severity of depression was positively related to sTNFR2 but not to sTNFR1. The underlying mechanisms involved with the triggering of TNF-a soluble receptors in depression are largely unclear. Previous

studies, however, have reported that severity of depressive symptoms was positively associated with pro-inflammatory cytokines (Loftis et al., 2008). Thus elevated sTNFR2 plasma levels could be related to this inflammatory status. Proteolytic cleavage (shedding) is a welldocumented mechanism to downregulate the cell response to TNF-a (Porteu and Nathan, 1990). It has been reported that downregulation of its receptors by TNF-a is due to the shedding of sTNFR2 and the internalization and shedding of sTNFR1, leading some authors to suggest the involvement of a TNF-a-dependent mechanism for sTNFR1 and a different mechanism, non-directly related to TNF-a, for sTNFR2 (Granell et al., 2004). Considering that its modulation has been related with other inflammatory pathway it was speculated that sTNFR2 could be more dependent on enhanced pro-inflammatory processes and subsequently more sensitive to depression severity.

Another interesting finding of the present study was that sTNFR1 levels were predicted by age and PTSD-like symptoms. The present data are in accordance with previous work indicating an age-related increase in sTNFR1 (Hasegawa et al., 2000). Moreover, the positive correlation observed between sTNFR1 and PTSD-like symptom severity is consistent with a low-grade systemic pro-inflammatory state directly related to PTSD symptom levels (von Kanel et al., 2007). We used PCL-C, however, to evaluate PTSD-like symptoms, and it is important to highlight that such a scale does not always specifically measure PTSD symptoms: instead depressive symptoms are also included. Therefore such findings could be the result of overlapping depressive symptoms that are part of the PCL-C scale. Future cross-sectional and longitudinal studies should take these variables into consideration to correctly assess soluble TNF-a receptors in MDD.

There are some limitations in the present study to be discussed. First, the stringent inclusion/exclusion criteria limited the sample size but yielded strictly healthy control subjects. It should be noted, however, that it was very difficult to recruit healthy controls from low socioeconomic status with no past or current psychopathology who did not report childhood maltreatment. The sample sizes, however, are in line with a previous study that measured TNF-a levels in MDD (Dome et al., 2008). Replication with large samples and longitudinal follow up is needed to overcome this limitation. Second, MDD participants were using antidepressants and it was not possible to discard the effects of medication on soluble TNF-a receptors or TNF-a levels. Indeed, the lack of detection of TNF-a could be related to high levels of soluble forms of its receptors present in plasma, which could bind circulating TNF-a and interfere with its detection (Granell et al., 2004). Taking into consideration that antidepressant treatment reduces TNF-a levels in MDD subjects (Himmerich et al., 2006), it is possible that this effect could be related to the increased level of TNF-a soluble receptors. Despite such considerations it should be noted that it is extremely difficult to investigate non-medicated patients who have recurrent MDD.

Conclusion

Taking into account the limitations described in the previous section, the present results suggest that sTNFR1 and sTNFR2 could be reliable markers of TNF-a activity in MDD. Moreover, particular roles of each soluble TNF-a receptors should be investigated. Specifically the complete understanding of mechanisms involved in the shedding of sTNFR2 and the internalization and shedding of sTNFR1, in addition to a better understanding of differential signals by TNFR1 and TNFR2 in neurons, is essential in order to develop new effective strategies. Although interesting and important studies have been per-

formed on anti-inflammatory treatments for depressive symptoms, particularly using celecoxib (Muller et al., 2006, Nery et al., 2008), future studies targeting TNF-a activity through sTNFR1 and sTNFR2 may be promising.

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Table 1.

	Depression (n = 30)		Control Subjects (n=19)		
	mean	SD ±	mean	SD ±	*p*
Age (years)	39.21	8.56	37.36	5.48	n.s.
BMI (kg/m^2)	26.89	2.86	25.54	2.50	n.s.
Education (years)	8.36	3.74	8.79	2.54	n.s.
Social Status †	63.75	6.95	62.57	4.79	n.s.
PCL-L scores	55.66	14.27	22.92	5.39	< .0001
BDI scores	28.54	11.14	4.78	3.92	< .0001
CTQ scores	58.36	18.32	41.71	10.62	< .0001

Figure 1.

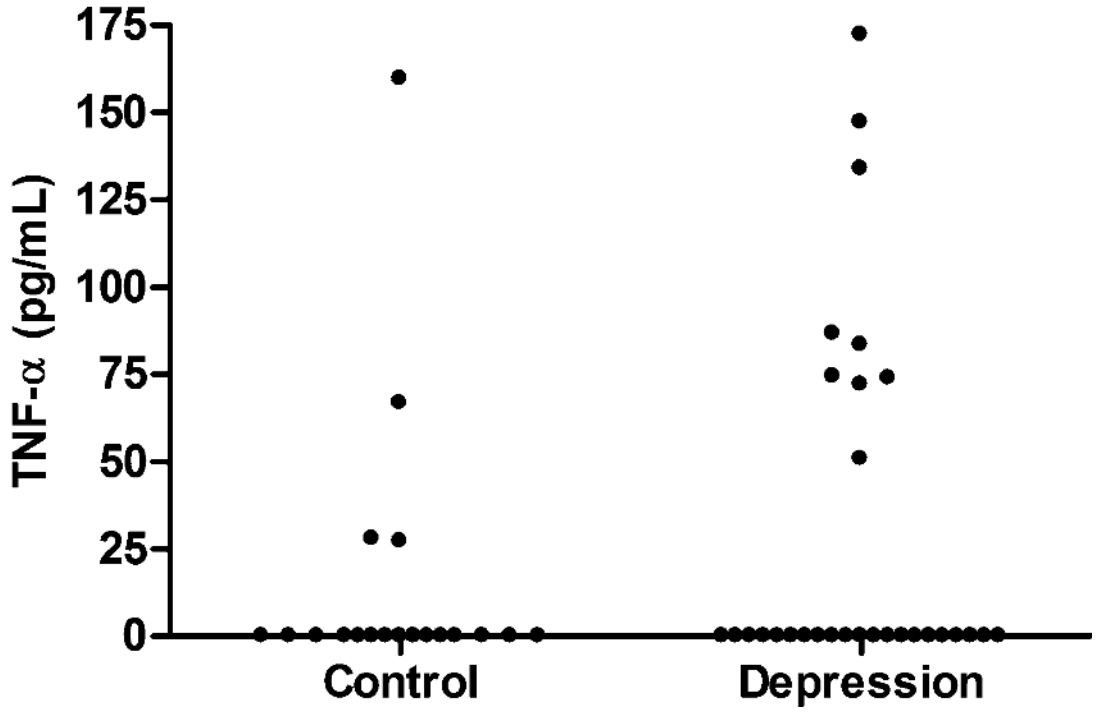

Figure 2.

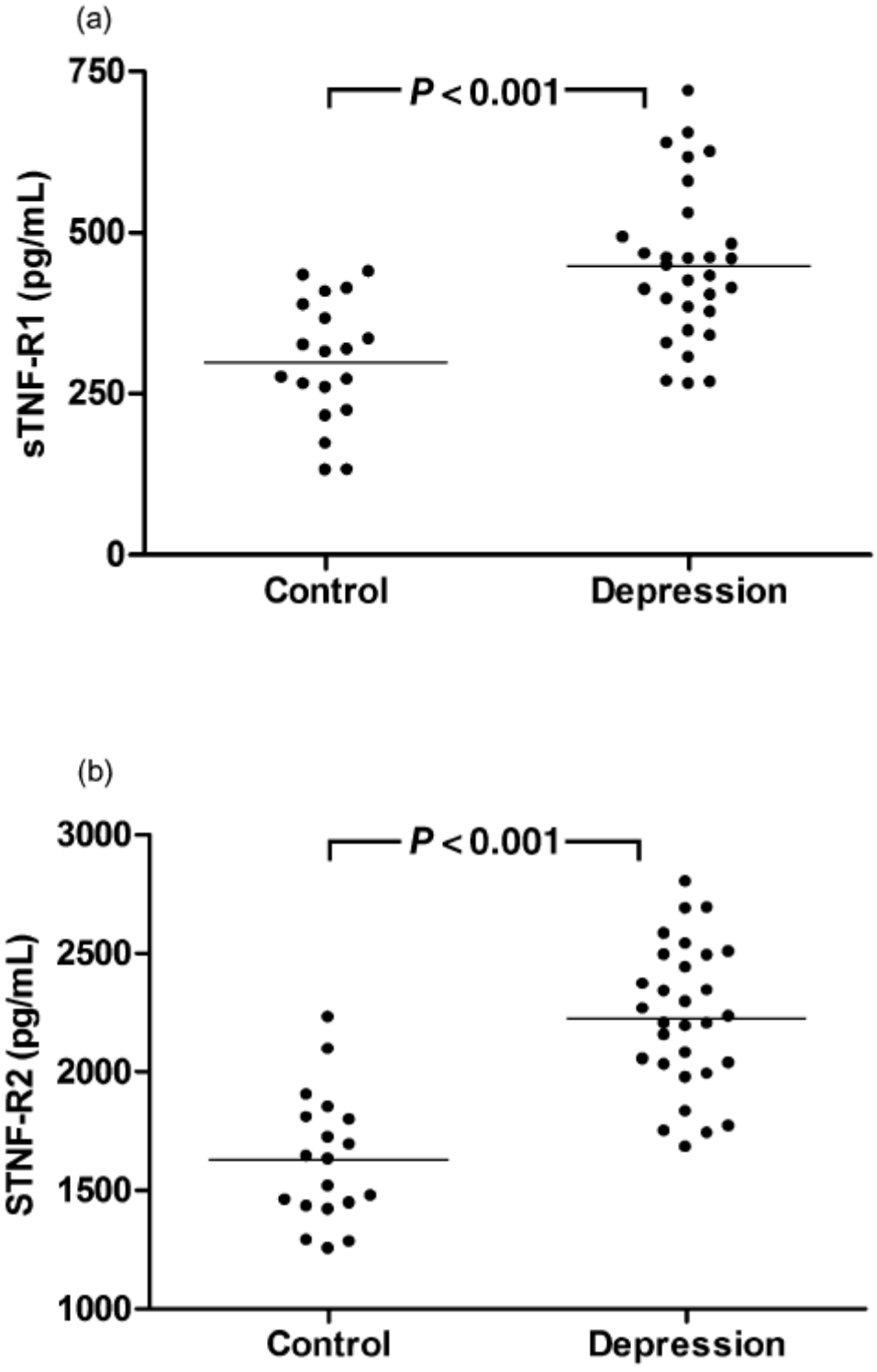
(a)
750
500
250
0
sTNF-R1 (pg/mL)
P < 0.001
Control
Depression
(b)
3000
2500
2000
1500
1000
STNF-R2 (pg/mL)
P < 0.001
Control
Depression

TABLE LEGENDS

Table 1. Subject characteristics. †Hollingshead's Index of Social Status. Statistical analysis was conducted using Student's *t*-Test. BDI, Beck Depression Inventory; BMI, body mass index; CTQ, Childhood Trauma Questionnaire; PCL-C, Post-Traumatic Stress Disorder Checklist–Civilian Version.

FIGURE LEGENDS

Figure 1. Plasma concentration of tumor necrosis factor-a (TNF-a) in outpatients with major depressive disorder (depression, $n = 30$) and healthy participants (control, $n = 19$).

Figure 2. Plasma concentration of (a) sTNFR1 and (b) sTNFR2 in outpatients with major depressive disorder (depression, $n = 30$) and healthy participants (control, $n = 19$). Horizontal lines indicate mean values. Statistical analysis (ANCOVA) was conducted using as covariates: age, depression severity, childhood abuse and neglect and post-traumatic stress disorder-like symptoms.

2.4 ARTIGO CIENTÍFICO

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Interplay between Neuroimmunoendocrine Systems during Post-Traumatic Stress Disorder: A Minireview

Moisés E. Bauer [b,c] , Andréia Wieck [c], Rodrigo Pestana Lopes [c,e], Antonio L. Teixeira [d] and Rodrigo Grassi-Oliveira [a]

[a]Faculty of Psychology, PUCRS, Porto Alegre, Brazil;

[b]Faculty of Biosciences, PUCRS, Porto Alegre, Brazil;

[c]Institute of Biomedical Research, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre, Brazil;

[d]Department of Internal Medicine, School of Medicine, Federal University of Minas Gerais (UFMG), Belo Horizonte, Brazil;

[e]BD Biosciences, São Paulo, Brazil.

Key words: Post-traumatic stress disorder, cortisol, hypocortisolism, cytokines, inflammation, lymphocytes.

Corresponding author: Dr. Moisés E. Bauer, Instituto de Pesquisas Biomédicas, Av. Ipiranga 6690, 2º andar. P.O. Box 1429. Porto Alegre, RS 90.610-000, Brazil. Email: mebauer@pucrs.br

ABSTRACT

Early life stress has been suggested to mediate vulnerability to affective disorders. Traumatic events experienced in childhood such as sexual abuse and/or physical neglect may lead to psychiatric diseases in adult life, including post-traumatic stress disorder (PTSD). Previous studies have focused on adult traumatic events and very little is known regarding the long-term physiological effects of early life stress. Here, we review the complex interplay between most important cognitive, neuroendocrine and immunological changes reported in PTSD, focusing on long-term implications of childhood maltreatment. PTSD has been associated with significant biological changes related to impaired cognitive functions, attenuated hypothalamic-pituitary-adrenal (HPA) axis function (hypocortisolism) and activation of innate immune responses (low-grade inflammation).

Introduction

Early life stress has been suggested to mediate vulnerability to psychopathology such as major depression and post-traumatic stress disorder (PTSD). Previous studies have mainly focused on adult traumatic events and very little is known regarding the long-term physiological effects of early life stress. Here, we review the complex interplay between cognitive, neuroendocrine and immunological changes reported in PTSD, focusing on long-term implications of childhood maltreatment. We discuss that many biological changes observed during PTSD could be attributable to insufficiency of glucocorticoids signaling.

Impaired HPA Axis Function

There is a general consensus that PTSD is associated with hypocortisolism. This finding may constitute a paradox because ever since the seminal studies by Selye (1936), stress has been associated with activation of the HPA axis with increasing cortisol levels. Indeed, cortisol hypersecretion has widely been used to define states of stress in human studies. Recently, hypocortisolism has also been observed in patients with burnout, physical complaints, chronic fatigue syndrome, fibromyalgia, chronic pelvic pain, asthma and others (Raison and Miller, 2003). These data suggest that hypocortisolism is not a specific phenomenon of PTSD.

However, chronically elevated levels of cortisol seem to exist in children who are currently living in adverse situations. Studies performed with maltreated children or who are diagnosed with PTSD show hypercortisolemia (De Bellis, 2002a). Girls exposed to sexual abuse show an impaired HPA axis function following pharmacological or nonpharmacological challenge tests. In line with such results, Heim et al. (Heim et al., 2000) found

that female adults with childhood sexual abuse had significantly higher cortisol and adrenocorticotropic hormone (ACTH) levels following exposure to acute psychosocial stress (Trier Social Stress Test, TSST) compared to controls. On the other hand, women with early childhood sexual abuse and PTSD had lower concentrations of cortisol during the afternoon hours (noon to 8 p.m.) compared with women with abuse without PTSD and women without abuse or PTSD (Bremner et al., 2007). It has been hypothesized that an early chronic increase of CRH would lead to downregulation of pituitary CRH receptors during life. This would be thus associated with adrenal insufficiency ('functional adrenalectomy'), and would ultimately explain the blunted cortisol levels found in women with a history of childhood abuse and PTSD.

Low-Grade Inflammation

The immune system is critically regulated by the glucocorticoids. In line with the lack of adequate glucocorticoid-mediated inhibition of immune responses, increasing evidence of immune activation has been reported in stress-related disorders characterized by hypocortisolism. There is growing evidence supporting the line between traumatic stress to a pro-inflammatory profile (Hoge et al., 2009), with increasing serum concentrations of TNF-a , IFN- g , IL-1b and IL-6. Recently, we observed that depressed patients with or without PTSD symptoms had higher soluble TNF receptor 2 levels in contrast to lower IL-2 and chemokine RANTES levels compared to healthy controls (Grassi-Oliveira et al., 2009). The low-grade inflammatory response has been linked to disease severity. PTSD patients seem to respond to mild everyday stressors with exaggerated anxiety responses that could potentially trigger the production of pro-inflammatory cytokines. It is also known that experimental psychosocial stress increase pro-inflammatory cytokines. The involvement of cyto-

kines in the pathophysiology of PTSD may involve changes of synaptic transmission, especially in hippocampal-amygdala structures, influencing various aspects of memory related to trauma.

The phenomenon of low-grade inflammation could be also involved with increased morbidity in PTSD. Peripheral administration of pro-inflammatory cytokines or increased levels observed during infections has been associated with changes in the patient's behavior known as 'sickness behavior'. The patient becomes irritable and exhibits increased sleep, depression, fatigue, decreased appetite and sexual drive. Pro-inflammatory cytokines have been implicated in this phenomenon. One clinical example of the role of cytokines in determining behavioral changes comes from studies evaluating the biological effects of IFN-g. Depression has been reported in up to 60% of patients with hepatitis C under treatment with IFN- g. The HPA axis may also have its activity enhanced by pro-inflammatory cytokines; however, a positive relationship between increased HPA axis activity and markers of immune activation is not well established.

Increased Cell-Mediated Immunity and Activation Phenotype

In addition to low-grade inflammation, patients with PTSD also reported important changes in cell-mediated immunity. For instance, NK cells, lymphocytes, T cells have been found particularly increased (Boscarino and Chang, 1999). The relative balance between Th1/Th2 immune responses seems to be balanced towards a potent Th1 immunity. Indeed, delayed-type hypersensitivity (DTH) was found enhanced in women with PTSD due to childhood sexual or physical abuse (Altemus et al., 2003).

The phenotype of lymphocytes is also altered in PTSD, suggesting an activation profile. Increased counts of activated T (CD2 + HLA – DR +), B (CD20 + CD23 +) and NK (CD16 + CD71 +) cell subpopulations in women with PTSD due to war displacement have been reported (Sabioncello et al., 2000). Lymphocytes expressing the late (CD71 +) but not the early CD25 +) activation marker were also elevated in displaced women. Another phenotype alteration is regarding the T cell memory profile, especially those associated with a history of childhood sexual abuse. An increased percentage of both central memory (CD45RA – CCR7 +) and effector memory (CD45RA – CCR7 –) T cell subsets has been observed in PTSD patients (Sommershof et al., 2009). Interestingly, Sommershof et al. [10] also observed a ~ 50% drop of regulatory T cells (CD4 + CD25 + FoxP3 +) in PTSD patients as compared to healthy controls. This substantial decline of regulatory T cells (Tregs) could bear the risk of excessive inflammation due to suboptimum control of immune responses and provide further support to the pro-inflammatory profile observed in patients with PTSD. Therefore, PTSD patients could be at risk for inflammatory disorders. Indeed, deficiency or dysfunction of Tregs in humans has been linked to several inflammatory and auto-immune diseases including multiple sclerosis, asthma, type 1 diabetes, psoriasis, and rheumatoid arthritis.

Altered Peripheral Sensitivity to Glucocorticoids

The effects of glucocorticoids on the immune system are mediated via both intracellular and membranebound glucocorticoid receptors (GRs). However, the functional effect of a stress hormone will depend on the sensitivity of the target tissue for that particular hormone. A reliable way of assessing the cross talk between peripheral hormones and the immune system is to determine the functional hormone action in specific target cells. We

have recently observed that dexamethasone (GR agonist) was less capable to suppress T cell proliferation of depressed women with long-standing PTSD symptoms due to childhood maltreatment, suggesting acquired steroid resistance. Conversely, it has also been shown that mononuclear cells from Bosnian war refugees with PTSD symptoms required less dexamethasone concentrations to inhibit cellular LPS-induced IL-6 and TNF- a secretion, suggesting increased sensitivity to glucocorticoids (Rohleder et al., 2004).

The magnitude of the biological effects of glucocorticoids is determined, among other factors, by the number and functionality of GRs. Yehuda et al. (Yehuda et al., 1991) reported increased densities of GR in peripheral lymphocytes of combat Vietnam veterans, without changes in cortisol levels. However, there are contradictory findings in the literature reporting lower GR densities with unaltered affinity for glucocorticoids. To what extent changes in glucocorticoid signaling in the immune system are related to fluctuations in cortisol levels or immune mediators (cytokines) remains to be determined.

Concluding Remarks

PTSD is associated with impaired homeostatic forces, leading to a significant allostatic load across the nervous, endocrine and immune systems. Hypocortisolism, impaired central and peripheral glucocorticoid signaling, low-grade inflammation and activation of cell-mediated immunity are commonly observed changes reported in PTSD. The constant stress experienced by PTSD patients may have important deleterious consequences and predispose the patient to stress-related disorders.

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Figure 1.

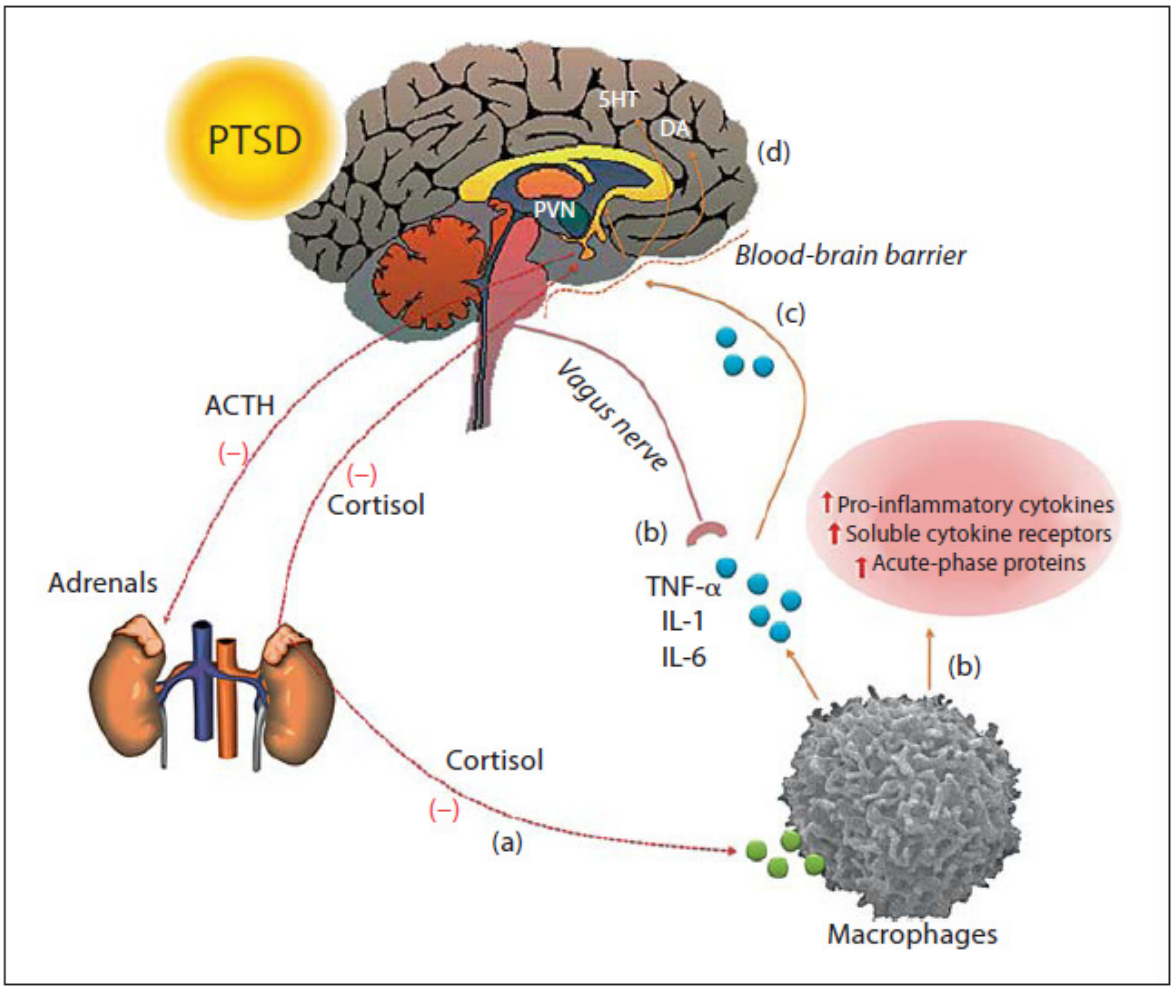
PTSD
5HT
DA
(d)
PVN
Blood-brain barrier
(c)
Vagus nerve
ACTH
(–)
(–)
Cortisol
(b)
↑ Pro-inflammatory cytokines
↑ Soluble cytokine receptors
↑ Acute-phase proteins
Adrenals
TNF-α
IL-1
IL-6
(b)
Cortisol
(–)
(a)
Macrophages

FIGURE LEGENDS

Figure 1. Neuroimmune interactions during PTSD. Hypocortisolism is associated with lack of adequate control of the immune system (a), leading to low-grade inflammation as indicated by increased levels of pro-inflammatory cytokines (b). These cytokines, in turn, access the brain via afferent fibers (e.g. vagus nerve) (b) or through leaky regions of the blood-brain barrier (c) or through active transport molecules. Once in the brain (d), cytokine signals participate in pathways known to be involved in the development of depression, including: (1) altered metabolism of relevant neurotransmitters such as serotonin (5HT) and dopamine (DA), and (2) activation of CRH in the paraventricular nucleus (PVN) and the subsequent production and/or release of ACTH and glucocorticoids (cortisol).

3. CONCLUSÃO E CONSIDERAÇÕES FINAIS

O estresse precoce na infância tem sido apontado como um possível agente influenciador na vulnerabilidade a doenças psicológicas como a Depressão Maior (DM) e o Transtorno do Estresse Pós-Traumático (TEPT) na vida adulta. A exposição precoce ao estresse na infância induziria modificações neurodesenvolvimentais ativadas pela natureza da experiência durante estágios críticos e sensíveis do desenvolvimento tanto do organismo (físico e mental) quanto das relações e interações sociais. Essas mudanças seriam na realidade respostas adaptativas frente a tais estressores na tentativa de habituar os indivíduos a elevados níveis de estresse (privação) durante esses períodos (Navalta et al., 2006). Estudos anteriores deram maior ênfase a avaliação de eventos traumáticos ocorridos na vida adulta e muito pouco se sabe a respeito dos impactos associados aos efeitos fisiológicos do estresse precoce na infância.

Maus-tratos infantis correspondem a um dos problemas sociais globais mais discutidos em meios especializados. As vítimas apresentam maior predisposição à depressão, ao uso abusivo de drogas lícitas e ilícitas, à obesidade, além de apresentarem comportamentos sexuais que aumentam a prevalência de doenças sexualmente transmissíveis e o risco de gravidez indesejada. Além das conseqüências ao desenvolvimento social, as conseqüências dos maus-tratos infantis também têm elevado impacto econômico às nações. Hospitalização, medicação, acompanhamento da saúde mental e outras despesas de longa duração são frequentemente necessárias, segundo a Organização Mundial da Saúde (WHO, 2010). Em relação a estudos de incidência e prevalência de maus-tratos e violência doméstica na infância, nosso país carece de

pesquisas na área. Conforme publicação do Fundo das Nações Unidas para a Infância, estima-se que aproximadamente 18 mil crianças e adolescentes sejam violentados diariamente (ISPCAN, 2006). Contudo, as conseqüências à saúde física e mental dos indivíduos vitimados, analisadas do ponto de vista das "cicatrizes biológicas" e de seus impactos, são pouco discutidas.

Nos trabalhos científicos que compõe esta tese, são apresentados e discutidos resultados de parâmetros neuroimunoendócrinos de mulheres deprimidas adultas, com e sem TEPT decorrente de abuso e negligência infantil (ANI), comparadas a um grupo de mulheres saudáveis, pareadas por índices de massa corporal, idade e estado social (nível educacional e faixa salarial). Os resultados desta tese demonstraram que mulheres adultas deprimidas e com TEPT decorrente de ANI apresentam importantes distúrbios fisiológicos que afetam o sistema endócrino e imune, além de ter reflexos sobre a memória. As pacientes tiveram redução da produção/secreção de cortisol e DHEA, hormônios com importantes e diversificados papéis na regulação e manutenção do equilíbrio do organismo; diminuição da capacidade proliferativa de linfócitos T mediante estímulo inespecífico, indicando um possível comprometimento da imunidade celular; maior resistência dos linfócitos T estimulados ao controle de proliferação por hormônios sintéticos e naturais, indicando tanto uma possível redução da capacidade natural do organismo de controlar respostas imunes mediadas por essas células quanto de uma menor resposta ao tratamento farmacológico com antiinflamatórios esteroidais; níveis elevados de receptores solúveis para TNF-a, indicando uma provável predisposição a reações inflamatórias mediadas por essa citocina; e redução dos níveis de BDNF, correlacionados com uma performance reduzida em testes de memória verbal.

Em suma, os dados aqui apresentados corroboram para que mais ações sociais sejam desenvolvidas de modo a prevenir a ocorrência de situações de maus-tratos na infância. Embora haja limitações associadas especialmente ao número amostral de participantes deste trabalho, foi possível comprovar que tais situações de exposição precoce ao estresse acarretam em conseqüências não só comportamentais, mas também fisiológicas e cognitivas de longa duração. Para dirimir questões que não puderam ser respondidas com base no delineamento e na limitação dos experimentos aqui apresentados, sugere-se que estudos longitudinais e com maior número amostral sejam realizados.

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ANEXO I

Termo de Consentimento Livre e Esclarecido

Está sendo realizada uma pesquisa na Faculdade de Psicologia da Pontifícia Universidade Católica de Porto Alegre (PUC-RS) sobre traumas ocorridos na infância e sua relação com problemas de memória, sono e hormônios do estresse na vida adulta.

A sua participação nesta pesquisa envolverá o fornecimento de informações sobre fatos da história da sua vida e fatos da sua situação atual. Estamos cientes que a natureza de algumas das informações que lhe solicitaremos poderá ser penosa e delicada, e não desejamos provocar maiores sofrimentos ao relembrá-las. Se você, mesmo assim, aceitar participar desta pesquisa, estaremos disponíveis para conversar sobre quaisquer sofrimentos despertados, e encaminhá-la, se necessário, para o atendimento psicológico ou médico adicional que se fizer necessário.

A pesquisa será realizada em dois dias no quinto andar do Hospital Materno Infantil Presidente Vargas, situado na Avenida Independência, 661 (o telefone do andar é 3289-3350, contato com Dr. Rodrigo Grassi). Assim, você terá que vir ao hospital duas vezes se desejar participar dessa pesquisa. Para isso os pesquisadores irão lhe fornecer a quantidade necessária de vale-transporte para o seu deslocamento até o hospital. No primeiro dia você terá que responder a uma entrevista com um médico sobre problemas psiquiátricos e deverá preencher três questionários que falam sobre sua infância, sintomas depressivos e sintomas de estresse. A duração é de aproximadamente 1 hora e 15 minutos. Após isso, será marcado o segundo encontro.

O segundo encontro sempre será realizado nas segundas, quartas ou sextas, na primeira hora da manhã (a partir das 8 horas) e terá duração de aproximadamente 1 hora. Nesse dia você fará um teste de memória que você terá que escutar uma lista de 120 palavras e depois tentar se lembrar delas. Além disso, você escutará duas histórias sendo solicitado que você repita o que se lembra por duas vezes. Nesse dia será necessário realizar um exame de sangue, onde serão colhidos cerca de 30 mL, e cuja picada da agulha não doerá mais do que uma coleta de sangue usual.

O exame de hormônio também é feito pela saliva (cuspe). Para isso, você será ensinada a coletar um pouco de saliva em um algodão e depois guardar no seu congelador, dentro de um tubinho. Essa coleta será feita em um único dia às 9h, 12h e 20 h. Se você tiver telefone residencial ligaremos nesses horários para instruí-la. Caso não possua telefone residencial, emprestaremos a você um telefone celular durante esse dia para que possamos falar com você nesses horários. Os pesquisadores irão visitar-lhe em casa para recolher o material da saliva e o celular (caso tenha sido emprestado).

Suas entrevistas, e as coletas de sangue para os exames, serão realizadas por profissionais da área da saúde, treinados para estes procedimentos.

Algumas pessoas receberão uma espécie de relógio que tem o objetivo de avaliar como anda o seu sono. Quem recebê-lo devera usar-lo no pulso por 24 horas. É muito importante que se tome muito cuidado com esse material, pois é bastante delicado. Quem usar deverá devolver para a pessoa que for buscar na sua casa, junto com o exame da saliva (cuspe) para a pessoa.

Seus registros médicos, e as informações que você fornecer serão tratados sempre confidencialmente (mantidos em segredo). Por outro lado, evitando que você seja

identificada, os resultados desse estudo poderão vir a serem publicados em revistas científicas, ou serem levados para discussão com outros profissionais da área da saúde.
Como participante desta pesquisa, você poderá desligar-se do estudo em qualquer momento, se assim o desejar, sem nenhum prejuízo de qualquer atendimento que esteja tendo, ou que possa vir a necessitar, do seu posto de saúde. Para seu conhecimento, os responsáveis pela pesquisa são o psiquiatra Rodrigo Grassi de Oliveira (telefone 3029-0654/9129-5992) e a psicóloga Profª. Dra. Lílian Milnitsky Stein (telefone 3320-3633), com os quais você poderá entrar em contato, sempre que desejar maiores esclarecimentos sobre o estudo que estará em andamento.
Declaro que estou ciente das informações acima e que concordo em participar desta pesquisa.

RG: _____

Nome do participante: _____

Assinatura do Pesquisador Responsável: _____

Assinatura da participante: _____

Porto Alegre, _____ de _____ de 2006.

ANEXO II

Aprovação no Comitê de Ética

PONTIFICIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
COMITÊ DE ÉTICA EM PESQUISA - CEP - PUCRS

Ofício 564/06-CEP Porto Alegre, 29 de maio de 2006.

Senhor(a) Pesquisador(a)

O Comitê de Ética em Pesquisa da PUCRS apreciou e aprovou seu protocolo de pesquisa registro CEP 06/03110, intitulado: **"Um estudo interdisciplinar sobre o impacto da violência infantil na vida adulta".**

Sua investigação está autorizada a partir da presente data.

Relatórios parciais e final da pesquisa devem ser encaminhados a este CEP.

Atenciosamente,

Prof. Dr. José Roberto Goldim
p/ COORDENADOR DO CEP-PUCRS

Ilmo(a) Sr(a)
Profa Lílian Milnitsky Stein
N/Universidade

ANEXO III

Carta de Aceite para Publicação de Artigo Científico na Neuroimmunomodulation

Ms No.: 201012003

Title: NEUROIMMUNOENDOCRINE INTERACTIONS IN PATIENTS WITH RECURRENT MAJOR DEPRESSION, INCREASED EARLY LIFE STRESS AND LONG-STANDING PTSD SYMPTOMS

Dear Dr. BAUER,

Thank you for submitting a revised version of the above mentioned manuscript to "Neuro-immunomodulation". It has now been evaluated by our Editors and reviewers.

Your paper has been accepted for publication on Feb 28th. I am now waiting for the Copy-right Transfer Form from you, before I can transmit the manuscript to the Production Dept.

Thank you in advance.

With kind regards,

Sandrine Maguire

Secretary to Professor Wilson Savino
NeuroImmunoModulation Editorial Office
S. Karger AG - Medical and Scientific Publishers
e: nim@karger.ch
f: +4161 3061434
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ANEXO IV

Registro dos Artigos Científicos Publicados

ARCHIVAL REPORTS

Low Plasma Brain-Derived Neurotrophic Factor and Childhood Physical Neglect Are Associated with Verbal Memory Impairment in Major Depression—A Preliminary Report

Rodrigo Grassi-Oliveira, Lilian Milnitsky Stein, Rodrigo Pestana Lopes, Antonio L. Teixeira, and Moisés Evandro Bauer

Background: Early life stress has been suggested to mediate vulnerability to affective disorders. Animal models of repeated maternal separation have shown reduced brain-derived neurotrophic factor (BDNF) levels in specific brain regions implicated with hypothalamic-pituitary-adrenal axis and memory formation. In addition, BDNF levels are also reduced in major depressive disorder (MDD) and bipolar disorder. The aim of this study was to investigate whether childhood physical neglect (CPN) and plasma BDNF levels would impact on memory performance in adult female subjects with recurrent major depression.

Methods: Recurrent female MDD outpatients with CPN (MDD + CPN, $n = 17$) and without CPN (MDD, $n = 17$) and healthy control subjects ($n = 15$) were assessed for plasma BDNF content and verbal memory performance. Memory was assessed through the logical memory component of the Weschler Memory Scale-Revised for immediate and delayed recall. Brain-derived neurotrophic factor was assessed with enzyme-linked immunosorbent assays (ELISAs).

Results: Major depressive disorder patients showed lower plasma BDNF concentrations than healthy control subjects ($p < .001$). Major depressive disorder + CPN had even lower BDNF levels compared with control subjects and MDD ($p < .05$). Brain-derived neurotrophic factor levels were negatively related to psychological morbidity and positively correlated to memory performance. Regression models showed that severity of self-reported CPN and low plasma BDNF predicted impairment on immediate verbal recall. Delayed recall impairment was predicted by severity of CPN and depression and memory retention by posttraumatic stress disorder (PTSD) severity symptoms.

Conclusions: Our data suggest that CPN and plasma BDNF are important factors associated with depression and verbal memory performance, particularly with encoding processes.

Key Words: Affective disorders, BDNF, childhood neglect, early life stress, major depression, memory

Early life stress has been suggested to mediate vulnerability to affective disorders including unipolar depression (1). A variety of studies has shown the close relationship between childhood abuse (particularly sexual and physical abuse) and neurobiological consequences in adult life (2). On the other hand, there are few studies that have examined the psychobiological consequences of neglect forms of childhood maltreatment (3).

Maternal separation (an animal model for early life stress in which rat pups are deprived of maternal contact once or repeatedly during the first postnatal weeks) could program widespread and lifelong changes in various transmitter systems that regulate the hypothalamus-pituitary-adrenal (HPA) axis (4). Some authors have suggested that repeated maternal separation could reduce brain-derived neurotrophic factor (BDNF) levels in specific brain regions (i.e., hippocampus) implicated with HPA axis and memory formation early in development (5). Despite any direct evidence implicated with maternal deprivation, BDNF activity, and memory performance, a reduction in BDNF activity in hippocampus of rats has been associated with a marked deficit in memory persistence (6). Brain-derived neurotrophic factor levels have been found to be reduced in major depression or bipolar disorder (7). However, even considering that in patients with mood disorders BDNF levels are lower than among those with a history of trauma (8), it is largely unknown to what extent BDNF levels are related to childhood physical neglect (CPN) and memory performance in major depression. It has been shown that specifically verbal declarative memory and small hippocampal volume could be related to childhood sexual abuse (9). Thus, the aim of this study was to investigate whether CPN and plasma BDNF levels would impact on verbal memory performance in adult female subjects with major depressive disorder (MDD).

Methods and Materials

Subjects and Clinical Assessment

Sixty MDD outpatients (all female subjects, 20–55 years) of an affective unit at the Hospital Presidente Vargas (Porto Alegre, Brazil) were interviewed by two well-trained clinical psychiatrists with the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) to confirm the diagnosis of recurrent MDD. A group of 17 outpatients with recurrent major depression without CPN (MDD) and 17 outpatients with recurrent major depression with

From the Faculty of Psychology (RG-O, LMS), Institute of Biomedical Research (RPL, MEB), and Faculty of Biosciences (MEB), Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre, Brazil; and Department of Internal Medicine (ALT), School of Medicine, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brazil.

Address reprint requests to Moisés E. Bauer, Ph.D., Instituto de Pesquisas Biomédicas, Av. Ipiranga 6690, 2° andar. PO Box 1429, Porto Alegre, RS 90.610-000, Brazil; E-mail: mebauer@pucrs.br.

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Regular Article

Increased soluble tumor necrosis factor-α receptors in patients with major depressive disorder

Rodrigo Grassi-Oliveira, MS, MD, PhD,[1]* Elisa Brietzke, MS, MD,[2] Júlio C. Pezzi, MD,[2] Rodrigo P. Lopes, MS,[3] Antonio L. Teixeira, MD, PhD[4] and Moisés E. Bauer, PhD[3]

[1]*Postgraduate Program of Psychology, Pontifical Catholic University of Rio Grande do Sul,* [2]*Psychiatry Department of Federal University of Rio Grande do Sul,* [3]*Institute of Biomedical Research, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil and* [4]*Department of Internal Medicine, Federal University of Minas Gerais, Belo Horizonte, Brazil*

Aim: Several lines of evidence suggest that major depressive disorder is associated with an inflammatory status. Tumor necrosis factor-α has been investigated as a potential molecular target in mood disorders. Tumor necrosis factor-α exerts its activity through binding to specific cell membrane receptors named as TNFR1 and TNFR2. The aim of the present study was to investigate soluble plasma TNFR1 (sTNFR1) and TNFR2 levels (sTNFR2) in major depressive disorder patients.

Methods: Female outpatients with major depressive disorder ($n = 30$) were compared with a healthy control group ($n = 19$). Severity of depressive symptoms was evaluated on Beck Depression Inventory; post-traumatic stress disorder (PTSD) symptoms were evaluated on PTSD Checklist–Civilian Version; and childhood abuse and neglect on the Childhood Trauma Questionnaire. Plasma tumor necrosis factor-α and its soluble receptors were measured by ELISA.

Results: Patients had no changes in tumor necrosis factor-α concentrations but did have increased sTNFR1 ($P < 0.001$) and sTNFR2 ($P < 0.001$) levels compared to controls. Plasma level of sTNFR1 was positively predicted by age ($B = 0.25$, $P = 0.05$) and PTSD-like symptoms ($B = 0.41$, $P = 0.002$) and plasma levels of sTNFR2 by depression severity ($B = 0.67$, $P < 0.001$).

Conclusions: Soluble tumor necrosis factor-α receptors could be reliable markers of inflammatory activity in major depression.

Key words: biological markers, child abuse, cytokines, mood disorder, inflammation.

RECENT STUDIES REPORT that patients with major depressive disorder (MDD) show activated inflammatory status, with increased pro-inflammatory cytokines, acute-phase proteins, and increased expression of chemokines and adhesion molecules.[1–3] Tumor necrosis factor-α (TNF-α) has been investigated as a potential molecular target in mood disorders,[4] and clinical improvement was associated with decreased serum levels.[5,6] In addition, specific depressive symptoms (e.g. suicidal behavior) were associated with increased cytokine levels.[7]

Tumor necrosis factor-α exerts its main effects by binding two specific receptors, TNFR1 (55 kDa) and TNFR2 (75 kDa).[8] The soluble forms of the TNF-α receptors (sTNFR1 and sTNFR2), which represent the extracellular portions of membrane-associated TNF-α receptors, play a role as modulators of the biological TNF-α activity.[9] The binding of TNF-α to TNFR1 leads to recruitment of associated death domain protein-mediated apoptosis and nuclear factor-kappa B

*Correspondence: Rodrigo Grassi-Oliveira, MS, MD, PhD, Av. Ipiranga, 6681, prédio 11, sala 933, Porto Alegre, RS 90619-900, Brazil.
Email: rodrigo_grassi@terra.com.br
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Interplay between Neuroimmunoendocrine Systems during Post-Traumatic Stress Disorder: A Minireview

Moisés E. Bauer[b, c] Andréa Wieck[c] Rodrigo P. Lopes[c, e] Antonio L. Teixeira[d] Rodrigo Grassi-Oliveira[a]

[a]Faculty of Psychology, [b]Faculty of Biosciences, and [c]Institute of Biomedical Research, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre, [d]Department of Internal Medicine, School of Medicine, Federal University of Minas Gerais (UFMG), Belo Horizonte, and [e]BD Biosciences, R. Alexandre Dumas, 1976 – Chácara Santo Antônio, São Paulo, Brazil

Key Words
Post-traumatic stress disorder · Cortisol · Hypocortisolism · Cytokines · Inflammation · Lymphocytes

Abstract
Early life stress has been suggested to mediate vulnerability to affective disorders. Traumatic events experienced in childhood such as sexual abuse and/or physical neglect may lead to psychiatric diseases in adult life, including post-traumatic stress disorder (PTSD). Previous studies have focused on adult traumatic events and very little is known regarding the long-term physiological effects of early life stress. Here, we review the complex interplay between most important cognitive, neuroendocrine and immunological changes reported in PTSD, focusing on long-term implications of childhood maltreatment. PTSD has been associated with significant biological changes related to impaired cognitive functions, attenuated hypothalamic-pituitary-adrenal (HPA) axis function (hypocortisolism) and activation of innate immune responses (low-grade inflammation).

Introduction

Early life stress has been suggested to mediate vulnerability to psychopathology such as major depression and post-traumatic stress disorder (PTSD). Previous studies have mainly focused on adult traumatic events and very little is known regarding the long-term physiological effects of early life stress. Here, we review the complex interplay between cognitive, neuroendocrine and immunological changes reported in PTSD, focusing on long-term implications of childhood maltreatment. We discuss that many biological changes observed during PTSD could be attributable to insufficiency of glucocorticoid signaling.

Impaired HPA Axis Function

There is a general consensus that PTSD is associated with hypocortisolism. This finding may constitute a paradox because ever since the seminal studies by Selye (1936), stress has been associated with activation of the HPA axis with increasing cortisol levels. Indeed, cortisol hypersecretion has widely been used to define states of stress in human studies. Recently, hypocortisolism has

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E-Mail karger@karger.ch
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Accessible online at:
www.karger.com/nim

Dr. Moisés E. Bauer
Laboratory of Cellular and Molecular Immunology, Institute of Biomedical Research
PUCRS, Av. Ipiranga 6690, 2° andar, PO Box 1429
Porto Alegre, RS 90610-000 (Brazil)
Tel. +55 51 3320 3000, ext. 2725, Fax +55 51 3320 3312, E-Mail mebauer@pucrs.br