Luciana Maria Silva de Seixas Maia

L-ARGININA FACILITA A DEPRESSÃO ALASTRANTE CORTICAL, DE FORMA DEPENDENTE DA DOSE, EM RATOS NUTRIDOS E PRECOCEMENTE DESNUTRIDOS

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Tese apresentada ao Programa de Pós-Graduação em Nutrição do Centro de Ciências da Saúde da Universidade Federal de Pernambuco, para obtenção do título de Doutor em Nutrição

Orientador: Dr. Rubem Carlos Araújo Guedes

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Banca examinadora:

Ana Paula Rocha de Melo – Drª. em Nutrição - UFPE

Ângela Amâncio dos Santos – Drª. em Nutrição - UFPE

Belmira Lara da Silveira Andrade da Costa - Drª. em Ciências – UFRJ

Leon Cintra McGlone – Dr. em Ciências – UNAM - México

Pilar Durán Hernández - Drª. em Ciências (Neurobiologia) – UNAM - México

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À minha família

Ao professor Rubem

Às minhas amigas Ângela e Marília

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O QUE É, O QUE É?

Composição: Gonzaguinha

É a vida?
E a vida o que é diga lá,
meu irmão?
Ela é a batida de um coração?
Ela é uma doce ilusão?
Mas e a vida?
Ela é maravilha ou é sofrimento?
Ela é alegria ou lamento?
O que é, o que é meu irmão?

Há quem fale que a vida da gente
É um nada no mundo
É uma gota, e um tempo
Que nem dá um segundo
Há quem fale que é um divino
Mistério profundo
É o sopro do criador
Numa atitude repleta de amor
Você diz que é luta e prazer
Ele diz que a vida é viver
Ela diz que o melhor é morrer
Pois amada não é
E o verbo sofrer

Eu só sei que confio na moça
E na moça ponho a força da fé
Somos nós que fazemos a vida
Como der ou puder ou quiser

Sempre desejada
Por mais que esteja errada
Ninguém quer a morte
Só saúde e sorte

E a pergunta roda
E a cabeça agita
Fico com a pureza da resposta
Das crianças
É a vida, é bonita e é bonita

Viver,
e não ter a vergonha de ser feliz
Cantar (e cantar e cantar)
a beleza de ser
Um eterno aprendiz
Eu sei
que a vida devia ser bem melhor
E será
Mas isto não impede que eu repita
É bonita, é bonita e é bonita

RESUMO

A L-Arginina atua como precursor do óxido nítrico, que possui várias funções no sistema nervoso, e cuja síntese pode ser modulada pela ingestão de L-Arginina. Em trabalho anterior deste laboratório (Frazão, 2004), demonstrou-se que a administração de 300mg/kg/dia de L-Arginina durante o aleitamento facilita, em animais bem nutridos adultos (90-120 dias), a propagação do fenômeno da depressão alastrante cortical (DAC). Neste trabalho, ratos lactentes, nutridos e desnutridos, foram tratados por gavagem (do 7º ao 28º dia de vida), com L-Arginina, em três doses (150, 300 e 450mg/kg/dia). Aos 30-40 dias, estudou-se o impacto desse tratamento sobre o peso corporal e encefálico, bem como sobre a propagação da DAC. Em comparação com dois grupos controles (um, tratado por gavagem com água e outro, sem tratamento – "ingênuo"), a administração de L-Arginina, nos animais bem nutridos, aumentou a velocidade de propagação da DAC, e este efeito variou positivamente com as três doses empregadas. Nos desnutridos, a velocidade da DAC aumentou apenas no grupo tratado com a dose mais alta (450mg/Kg/dia). Os dados indicam que a administração de L-Arginina durante o período de desenvolvimento facilita a propagação da DAC, de forma dependente da dose e também do estado nutricional precoce. Sugere-se que, nesse efeito, esteja envolvida a modulação da síntese de óxido nítrico conseqüente à administração de L-Arginina.

Palavras Chave: Arginina, Desnutrição, Depressão alastrante cortical, Desenvolvimento cerebral, Oxido nítrico.

ABSTRACT

L-Arginine acts as the precursor for the synthesis of nitric oxide (NO), a compound that has important functions in the nervous system. NO synthesis can be modulated by ingestion of L-Arginine. NO-mediated signaling seems to be involved in the phenomenon of cortical spreading depression (CSD). In a previous investigation (Frazão, 2004), it was demonstrated that the administration of 300mg/kg/day of L-Arginine during the suckling period facilitates, in adult well nourished animals (90-110 days), the propagation of the phenomenon of cortical spreading depression (CSD). In this study, young, well-nourished and malnourished rats were treated, by gavage, with 150, 300 or 450mg/kg/d of L-Arginine from postnatal days 7-28, and body- and brain weights, as well as CSD propagation, were analyzed at 30-40d. Compared with two control groups (one, treated per gavage with water and another one, without treatment - "naive" group), the well-nourished L-Arginine treated groups dose-dependently displayed higher CSD-velocities ($P<0.05$). However, in the malnourished rats only the highest L-Arginine-dose (450mg/kg/d) increased CSD velocities. The mean±sd CSD-velocities (in mm/min) were: for well-nourished rats, 3.77±0.15, 3.78±0.23, 4.03±0.16, 4.36±0.19 and 4.41±0.26, in the naïve-, water-controls, 150, 300 and 450 mg/kg/d ARG-groups, respectively. For the same conditions in the malnourished rats, the velocities were 4.18±0.13, 4.22±0.09, 4.24±0.10, 4.27±0.21 and 4.64±0.22, respectively. Results demonstrate a CSD-facilitation by L-Arginine treatment during the fast brain development period, and this effect is dependent on the dose- and on the nutrition-status early-in-life. It is suggested that the modulation of nitric oxide synthesis by the treatment with L-Arginine could be involved on the here reported CSD facilitation.

Key word: Argnine, Brain development, Cortical sprerading depression, Malnutrition, Nitric oxide.

LISTA DE ABREVIATURAS E SIGLAS

1. ANOVA – Análise de variância
2. CAPES – Coordenação de aperfeiçoamento de pessoal de nível superior
3. DAC – Depressão alastrante cortical
4. ECOG – Eletrocorticograma
5. eSON – Sintase do óxido nítrico endotelial
6. iSON – Sintase do óxido nítrico induzida
7. KCl – Cloreto de potássio
8. LAFINNT – Laboratório de fisiologia da nutrição Naíde Teodósio
9. L-ARG – L-Arginina
10. N12 – Ninhada de 12 filhotes por mãe
11. N6 – Ninhada de 6 filhotes por mãe
12. NADPH-d – Nicotinamida adenina di-nucleotídio fosfato diaforase
13. NAIVE – Animais que não sofreram tratamento
14. nSON – Sintase do óxido nítrico neuronal
15. ON – Óxido nítrico
16. PTZ – Pentilenotetrazol
17. SON – Sintase do óxido nítrico
18. UFPE – Universidade Federal de Pernambuco

SUMÁRIO

1. INTRODUÇÃO

A arginina é um aminoácido do tipo condicionalmente essencial, isto é, crianças e animais recém-nascidos não têm condições de sintetizá-lo e por isso, nessas fases da vida, ele tem de ser fornecido através de alimentos ricos em proteínas (Mahan e Escott-Sump, 2005). Como precursor de importantes moléculas biológicas, a arginina exibe versatilidade metabólica e regulatória (Nieves jr. e Langkamp-Henken, 2002). A L-Arginina (L-Arg) dá origem ao óxido nítrico que atua como neurotransmissor (Garthwaite et al., 1988), além de ter importante papel na regulação do fluxo sanguíneo (Moncada et al., 1989) e na plasticidade sináptica (Paakkari e Lindsberg, 1995; Maia et al., 2006).

Recentemente, Frazão (2004) demonstrou que a administração diária de 300mg/kg/dia de L-Arginina durante o aleitamento facilita, na idade adulta, a propagação do fenômeno da depressão alastrante cortical (DAC), que também é influenciada pela desnutrição (Rocha-de-Melo e Guedes, 1997; Rocha-de-Melo et al., 2006). Nestes trabalhos, em ratos, ficou provado que tanto a desnutrição pela "dieta básica regional", como pelo aumento do tamanho da ninhada foram eficientes para reduzir os pesos corporais e encefálicos, bem como facilitar a propagação da DAC. Parece que este efeito é independente da quantidade de nitrogênio fornecida pela L-Arginina, visto que a suplementação com histidina (que fornece quantidades equivalentes de nitrogênio, mas nada tem a ver com a síntese do óxido nítrico) não reproduziu o mesmo efeito (Frazão, 2004).

Quando testada em ratos precocemente desnutridos, a L-Arginina provocou efeitos menos intensos sobre a DAC, em comparação com aqueles observados nos bem-nutridos (Frazão, 2004). Na avaliação histológica dos neurônios nicotinamida adenina di-nucleotídio fosfato diaforase (NADPH-d) positivos no córtex visual confirmou-se a resistência destes à desnutrição (Maia et al., 2006; Borba et al., 2000), mas eles se mostraram susceptíveis à L-Arginina, aumentando a arborização e as varicosidades dendríticas (Maia et al., 2006). Esses dados podem tem relevância nas relações entre nutrição e função visual humana. Funções básicas neurais, como o processamento da informação visual e a percepção da sensação correspondente, assim como as execuções de tarefas motoras dependentes da

visão, podem ser afetadas por mudanças do estado nutricional, capazes de alterar estruturas do córtex visual (Dowdeswell et al., 1995; Powls et al., 1997).

O estudo inicial de Frazão (2004), em *ratos adultos* tratados precocemente *com uma única dose de L-Arginina*, originou a linha de investigação que foi continuada pelo presente trabalho. Para isto, investigou-se, *em uma idade mais precoce* do que aquela estudada por Frazão, o impacto do tratamento com L-Arginina *em distintas doses ("curva dose-resposta")*, associado à desnutrição pelo aumento do tamanho da ninhada. Além disso, comparou-se os dados obtidos em animais jovens com aqueles dos animais adultos de Frazão, como será brevemente descrito na metodologia.

OBJETIVOS

2.1. GERAL: Investigar, em ratos recém-desmamados, os efeitos do tratamento prévio (no aleitamento) com diferentes doses de L-Arginina, associadas a condições favoráveis e desfavoráveis de lactação, sobre a evolução ponderal e a atividade elétrica cerebral.

2.2. ESPECÍFICOS:

- Investigar a evolução do peso corporal dos filhotes lactentes ao longo do seu desenvolvimento;
- Quando os filhotes forem desmamados, avaliar os efeitos do tratamento dietético precoce com L - Arginina sobre:
  - a suscetibilidade cortical à DAC, através de sua incidência e propagação;
  - o peso do encéfalo, obtido ao final do registro eletrofisiológico
- Adicionalmente, comparar os dados presentemente obtidos com aqueles dos animais de Frazão, (2004), que sofreram igual tratamento, porém foram avaliados na idade adulta.

HIPÓTESES

As hipóteses levantadas neste estudo são as de que:

- A administração de L-Arginina, a ratos lactentes, associada à manipulação do tamanho da ninhada induz alterações sobre a curva de peso e também aumenta a velocidade de propagação da Depressão Alastrante Cortical.
- Os efeitos acima postulados são dependentes da dose e do estado nutricional pregresso a que os lactentes foram submetidos.

2. REVISÃO DA LITERATURA

O organismo é constituído por vários sistemas fisiológicos e cada um deles necessita de tipos diferentes de nutrientes, com funções específicas, sendo então necessário uma dieta variada, equilibrada e harmônica para que o organismo tenha um bom funcionamento (Mahan & Escott-Stump, 2005). Compreender a relação da nutrição com o sistema nervoso é fundamental, visto que este tem importante papel na integração e coordenação de todas as funções do organismo (Guedes et al, 2007).

O período de desenvolvimento cerebral mais intenso varia para as diferentes espécies de mamíferos. No caso do homem, esse período é compreendido entre o terceiro trimestre de gestação e o segundo ou terceiro anos de vida, ou seja, inclui um período pré e outro pós-natal. No rato, assim como no cão, compreende apenas o período do aleitamento. Além disso, o cérebro como um todo parece não se desenvolver homogeneamente, apresentando ritmos de crescimento diferenciado para suas distintas partes. (Morgane et al., 1993, Levitsky et al, 1995).

A nutrição adequada, especialmente no início da vida, é crucial para um bom desenvolvimento do sistema nervoso (Guedes, 2005). Mesmo modificações em constituintes de um único nutriente podem afetar o desenvolvimento e funcionamento do sistema nervoso (Gietzen et al, 1998; El-Bachá et al, 1998). Um exemplo disto é o que ocorre com a L-Arginina. Aumentos na oferta desse aminoácido durante o desenvolvimento podem afetar, de forma marcante, o funcionamento do sistema nervoso central (Frazão, 2004; Maia et al, 2006)

Arginina, Óxido Nítrico e Funções Neurais

ARGININA

Crianças e outros mamíferos recém-nascidos não têm condições de sintetizar o aminoácido Arginina e por isso, ele é considerado do tipo condicionalmente essencial (Mahan e Escott-Sump, 2005). Nesta fase inicial da vida, a Arginina tem de ser fornecida através de alimentos ricos em proteínas. Além do leite materno, outros alimentos ricos em proteínas, e, portanto boas fontes de Arginina são: carnes, ovos, leite e seus derivados, além de peixes, amendoim e nozes (Böger, 2007). O seu requerimento, para o rato adulto (peso médio de 300g), pode ser estimado a partir do seu teor na dieta (4,5k/kg de dieta; Reeves et al., 1993). Considerando-se um consumo médio de 20g de dieta/rato/dia, chega-se à ingestão de 90 mg/dia, para um rato adulto.

Como precursor de importantes moléculas biológicas, a L-Arginina exibe versatilidade metabólica e regulatória (Nieves jr. e LangKamp-Henken, 2002). Tem importante papel sistêmico na regulação do fluxo sanguíneo (Moncada e col., 1989). Além disto estudos indicam que no sistema nervoso central, tem implicação na plasticidade sináptica (Paakkari e Lindsberg, 1995; Maia et al., 2006).

As rotas metabólicas da L-Arginina nos levam a perceber que este aminoácido está implicado no ciclo da uréia, na síntese de proteínas, creatinina e poliaminas, além de ser precursor do óxido nítrico (Nieves jr. e LangKamp-Henken, 2002).

Em humanos, geralmente, a L-Arginina é bem tolerada quando administrada por vias como a intraperitonial, a intravenosa ou a oral em quantidades de até 30 g. Em doses superiores a esta, por via oral, pode provocar vômitos e náuseas, e por via intravenosa irritação local e flebites devido à alta osmolaridade da solução (Böger e Bode-Böger, 2001). A absorção da L-Arginina ocorre rapidamente e quase completamente através da membrana da borda em escova dos enterócitos por meio do sistema de transporte ativo y+ (Böger e Bode-Böger, 2001, Wiesinger, 2001).

Sabe-se que o aumento ou a diminuição de um ou mais nutrientes na dieta é capaz de promover agravos ao indivíduo. No sistema nervoso o desequilíbrio de aminoácidos pode causar alterações neuroquímicas (Gietzen, 1993; Gietzen et al., 1998; Gietzen e Magrum, 2001). Há também relatos de que altas doses de L-Arginina dietética (até 50g/kg) durante a desnutrição, estão relacionadas com dificuldades de ganho de peso e aumento de mortalidade por infecção em ratos com desnutrição protéica (Peck et al., 1995). No entanto, é importante lembrar que o organismo tem grande capacidade de adaptação tentando buscar a homeostase. Assim, de maneira geral, o organismo parece ser capaz de manter níveis de aminoácidos próximos aos limites normais no cérebro e fígado, a despeito da variabilidade protéica da dieta (Colombo et al., 1992).

ARGININA E ÓXIDO NÍTRICO

Uma das principais rotas metabólicas de interesse para o nosso trabalho é aquela em que a L-Arginina dá origem ao óxido nítrico (ON). O ON é um gás solúvel, sintetizado pelas células endoteliais, macrófagos, e também neurônios (Bredt e Snyder, 1992). Evidências indicam o ON como sendo um mensageiro molecular em pelo menos três sistemas: leucócitos, onde media efeitos bactericidas e tumoricidas; vasos sanguíneos, onde apresenta atividade de fator relaxante derivado do endotélio, e como um constituinte neuronal com funções de neurotransmissor (Bredt e Snyder, 1992).

A L-Arginina é convertida a óxido nítrico e L-Citrulina, na presença do oxigênio, através da enzima sintase do óxido nítrico (SON), sendo para isto consumidos 5 elétrons. Existem três isoformas da SON; a nSON que é constitutiva e está predominantemente no tecido neuronal, a iSON que é induzida e está localizada em vários tecidos e ainda a eSON que também é constitutiva e se localiza no tecido endotelial (Dawson e Dawson, 1996; Flora Filho e Zilberstein, 2000).

A sintase do óxido nítrico tem sido co-localizada com a NADPH-d (Dawson e Dawson, 1996). Neurônios que contêm a enzima NADPH-d, formam, no córtex cerebral do rato, uma população de 1 a 2% das células neuronais. Eles foram histoquimicamente identificados como sendo marcados por uma cor azul escuro na presença do *nitroblue tetrazolium* (Bredt e Snyder, 1992; Franca et al., 1997; 2000). Estes neurônios são bastante resistentes a insultos neurotóxicos (Dawson e Dawson, 1996; Maia et al, 2006).

Dentre as várias funções do ON no organismo, sabe-se que ele atua como um neurotransmissor atípico (Garthwaite et al., 1988). Ele parece ser liberado de neurônios pós-sinápticos por meios não-vesiculares e atuar sobre os terminais pré-sinápticos, o que caracteriza o óxido nítrico como um mensageiro retrógrado. Sendo o ON uma molécula pequena, pode atravessar facilmente as membranas das células próximas ao local onde é produzido. Podendo difundir-se livremente, sua ação pode se distribuir ao longo de uma região pequena do tecido neural. No entanto, é degradado muito rapidamente (Bear et al, 2002).

O ON tem papel chave na morfogênese e na plasticidade sináptica (Snyder, 1992; Paakkari e Lindsberg, 1995; Dawson e Dawson, 1996). Tem sido proposto ser um modulador fisiológico da proliferação celular (Vilalobo, 2006). Atua na modulação do sistema hipotálamo-hipofisário (Kadekaro, 2004), no fenômeno da potenciação a longo prazo, no hipocampo, e na depressão a longo prazo, no cerebelo. Desta forma, ele é indiretamente capaz de modular sinapses que possam estar envolvidas em processos de aprendizagem e memória (Snyder, 1992; Dawson e Dawson, 1996).

Com relação ao papel fisiológico do ON no sistema nervoso, estudos em animais de laboratório indicaram que a SON está seletivamente diminuída no hipocampo e no tronco cerebral de animais mais velhos o que nos leva a inferir que alguns mecanismos regulados pelo ON nestas áreas podem estar relacionados com algumas desordens cerebrais em pessoas idosas (Mollace et al., 1995). O ON reage com um ânion superóxido que produz radicais livres, os quais são responsável por efeitos neurotóxicos. Em altas concentrações, o ON é tóxico e assim pode desencadear doenças neurodegenerativas, tais como a doença de Huntington e a de Alzheimer (Dawson e Dawson, 1996). Por outro lado, há indícios de que substâncias antioxidantes naturalmente presentes no organismo, tais como as vitamina C e E, provavelmente têm um papel importante na redução ou eliminação dos danos oxidativos produzidos pelo excesso de ON (McCann et al., 2005).

Se por um lado fica claro que o ON pode ser citotóxico, a depender da sua concentração, por outro postula-se que ele pode ter um efeito neuroprotetor, em certas condições (Pérez-Sereviano et al., 2002). A administração, por infusão, de doses agudas de L-Arg (300mg/Kg) após injúria cerebral traumática experimental foi capaz de aumentar o fluxo sanguíneo cerebral, reduzindo o dano neurológico (Cherian et al., 2003). Nesse estudo, os autores analisaram a janela temporal que vai até 48 horas após o dano, sendo observado o efeito protetor quando o referido aminoácido foi administrado de 5 min a 1 hora depois do dano. Durante o nosso trabalho de mestrado, tratamos ratos lactentes com a mesma dose de L-Arg, acima referida (via gavagem) e os estudamos quando adultos, com relação ao padrão histoquímico de neurônios corticais marcados com a técnica da NADPH-diaforase. Nessas condições, foi possível demonstrar aumento da arborização e do número de varicosidades de neurônios NADPH-d positivos no córtex visual (Maia et al., 2006). Resultados sugestivos de neuroproteção foram também encontrados em ratos previamente

injetados com aquela mesma dose de L-Arg (300 mg/kg; i.p.) e, em seguida, tratados com Pentilenotetrazol (PTZ; um modelo experimental de epilepsia). Esse efeito parece depender da idade do animal (Pereira-de-Vasconcelos et al., 1998; 2000).

O papel do ON nas crises convulsivas tem sido outra área de estudo que vem crescendo em investigações experimentais. Além do aspecto referido no parágrafo anterior, quanto a efeitos dependentes da idade, tem sido demonstrado que o hipocampo e o estriado apresentam respostas diferentes à L-Arg exógena na produção do óxido nítrico (Hara et al., 2004) e que iSON em excesso e a deficiência de eSON podem interagir em modelos animais de epileptogênese (Murashima et al., 2000). Analisados em conjunto, os trabalhos acima indicam que mais estudos são necessários para a melhor compreensão do papel da arginina na epileptogênese.

Outro aspecto importante que tem sido relatado é que a inibição da SON tem sido descrita como capaz de melhorar a eficácia clínica de antidepressivos serotoninérgicos (Harkin et al., 2003; 2004). Níveis elevados de ON parecem aumentar nos tecidos a liberação de neurotransmissores, visto que o óxido nítrico funciona como um modulador neuronal (Prast e Philippu, 2001). Desta forma, é possível que as concentrações endógenas de ON possam influenciar a eficácia terapêutica de antidepressivos serotoninérgicos usualmente prescritos na clínica médica, tais como a fluoxetina e a tianeptina.

A síntese do ON “in vivo” a partir da L-Arg pode ser prejudicada pela deficiência dietética protéica em geral ou, especificamente pela carência de arginina, uma vez que tais deficiências podem diminuir a concentração sérica desse aminoácido, bem como a excreção urinária de nitrato. O nitrato é o principal produto final estável do ON em animais e humanos; sua excreção através da urina tem sido usada como um indicador da síntese de ON (Wu et al., 1999).

Essa síntese pode também ser alterada pelo tipo de anestésico utilizado no trabalho experimental. Por exemplo, aumento drástico de ON foi identificado no cérebro de ratos sob anestesia com Halotano, enquanto que o Pentobarbital e o Hidrato de cloral produziram um aumento insignificante, porém a Ketamina diminuiu a produção do mesmo (Sjakste et al., 1999). Esses dados indicam a importância de se escolher adequadamente o tipo do anestésico em experimentos envolvendo a quantificação do ON.

DESNUTRIÇÃO, ARGININA E DEPRESSÃO ALASTRANTE CORTICAL

O crescimento e o desenvolvimento do sistema nervoso ocorrem com velocidades máximas nas fases iniciais da vida (Smart e Dobbing, 1971; Smart, 1990). Esses dois processos parecem se desenvolver em etapas pré-determinadas e inflexíveis, de sorte que se uma delas não ocorrer ou acontecer fora da janela temporal pré-programada, os danos poderão ser irreversíveis (Morgane 1993; Borba et al., 2000). A má nutrição, seja por excesso ou diminuição em um ou mais nutrientes, é um dos principais fatores não genéticos, que pode alterar negativamente esses processos (Morgane et al., 1992; 1993). Na realidade, o impacto da má nutrição sobre o sistema nervoso pode ser maior ou menor; depende do período em que a alteração nutricional acontecer (Smart e Dobbing, 1971; Rocha-de-Melo e Guedes, 1997), bem como de sua intensidade e duração (Morgane et al., 1993).

Estudos indicam que a desnutrição precoce pode acarretar seqüelas permanentes ou transitórias no sistema nervoso (Borba et al., 2000). Isto parece estar relacionado aos mecanismos de plasticidade cerebral (Bennett et al., 1964; Maia et al., 2006). As estruturas neurais que têm sido descritas como sendo mais afetadas pela desnutrição são o hipocampo, o cerebelo e o bulbo olfatório. Essas áreas terminam a sua formação logo após o nascimento. Assim, elas estão mais sujeitas aos danos provocados por agravos nutricionais que possam ocorrer durante esse período (Morgane et al., 1992; 1993, Levitsky e Strupp, 1995).

Alterações nutricionais podem ocasionar mudanças neuroanatômicas, neuroquímicas e comportamentais, repercutindo na capacidade de memória, cognição e na motivação do indivíduo (Barret e Radke-Yarrow, 1985; Hack et al, 1991; Strupp e Levitsky, 1995; Ranade et al., 2008). Os processos de memória e aprendizagem dependem de numerosas interações de neurotransmissores que derivam de sistemas bioquímicos e metabólicos em várias partes do cérebro (Morgane et al., 1993). Além disso, a desnutrição parece afetar diferencialmente a atividade de neurotransmissores cerebrais. Por exemplo, as transmissões GABAérgica e colinérgica podem ser reduzidas pela deficiência nutricional,

contrastando com a ativação que tem sido observada nos sistemas serotoninérgico e catecolaminérgico (Wiggins et al, 1984).

As mudanças desencadeadas pela desnutrição durante o desenvolvimento neural podem levar à maior densidade de empacotamento celular, ao menor número de células, à diminuição de lipídios, e conseqüente diminuição da mielinização, além de alterar a atividade de enzimas (Dobbing, 1970; Dobbing e Smart, 1974; Krigman e Hogan, 1976).

Em todo o mundo, especialmente em países em desenvolvimento como o Brasil, a desnutrição protéico-energética continua sendo um problema de saúde pública, apesar da melhoria demonstrada pelos indicadores populacionais (Monteiro, 1997; Onis et al., 2000). No entanto, é importante lembrar que há outros graves problemas de saúde pública no que diz respeito à deficiência de nutrientes específicos, tais como a hipovitaminose A e a anemia ferropriva (Barreto e Carmo, 1994). Além disto, a alimentação desequilibrada de algumas populações está fazendo com que ocorra um aumento da incidência de obesidade (Jakoby, 2004). Em todos os aspectos acima referidos pode haver implicações ao nível de formação e desenvolvimento do sistema nervoso central.

A partir das evidências até aqui comentadas, torna-se claro que estudar as implicações dos agravos nutricionais ao sistema nervoso, não é uma tarefa fácil. Tem-se que fazer uso de métodos e técnicas específicas na tentativa de compreender a estrutura e o funcionamento cerebrais. Para testar a ação de nutrientes específicos sobre o sistema nervoso, o uso de modelos experimentais é de grande importância. Um desses modelos é constituído pelo fenômeno da "depressão alastrante da atividade elétrica cerebral" (DAC) que será descrito neste trabalho, com vistas ao seu uso em estudos futuros.

A DAC foi descrita, pela primeira vez, pelo pesquisador brasileiro Aristides Leão, em 1944**.** Ele observou que a estimulação elétrica, química ou mecânica de uma área restrita da superfície cortical provocava uma resposta reversível nesse tecido. Essa resposta caracterizava-se por uma intensa diminuição (depressão) da atividade elétrica espontânea ou evocada. A duração da depressão das oscilações elétricas é longa, cerca de 1 a 2 minutos, o que a distingue de outros fenômenos eletrofisiológicos, como o potencial de ação, com duração da ordem de milissegundos. Uma vez deflagrada, a DAC se propaga (alastra) de maneira concêntrica e lentamente para outras áreas corticais. No rato, por exemplo, a sua velocidade de propagação é de 3 a 4 mm/min. Cerca de 10 a 15 minutos

após a deflagração da DAC, a atividade elétrica cortical recupera-se completamente, o que demonstra ser a DAC um fenômeno reversível (Leão, 1944a).

Durante a DAC, além da depressão eletrocorticográfica, ocorrem várias modificações em outros parâmetros do tecido, tais como: dilatação dos vasos sanguíneos da pia-máter (Leão, 1944b), aparecimento de uma variação lenta de voltagem medida contra um ponto de potencial elétrico fixo (Leão, 1947), variação da quantidade de água e das concentrações de íons no espaço intersticial (Hansen e Olsen, 1980), dentre outros.

Os mecanismos subjacentes ao fenômeno da DAC ainda não estão completamente esclarecidos. A presença de ondas eletroencefalográficas com características que parecem de natureza epileptiforme levou à idéia de que a DAC teria algo em comum com o fenômeno epiléptico, em termos de mecanismos (Leão, 1944). Além da epilepsia, a DAC também pode ser usada como um modelo experimental que pode dar subsídios para melhorar a compreensão de processos que levam a outras patologias neurais, como a enxaqueca clássica. Ambos os fenômenos (a DAC e a enxaqueca clássica) apresentam alterações vasculares semelhantes e as velocidades de propagação da DAC são parecidas com as de expansão, no campo visual, dos escotomas cintilantes relatados pelos doentes, pouco antes de sofrer um episódio da doença (Lauritzen, 1987; 2001).

No Laboratório de Fisiologia da Nutrição Naíde Teodósio (LAFINNT) investiga-se até que ponto animais submetidos a diversas condições experimentais, especialmente as relacionadas à nutrição, podem sofrer alterações nos aspectos estruturais e funcionais do sistema nervoso, e como essas modificações influenciam a susceptibilidade do tecido cortical à DAC. Isto é possível através de estudo da incidência e da propagação da DAC. Dessa forma, a DAC tem sido usada como um indicador de funcionamento e desenvolvimento do Sistema Nervoso Central (Guedes, 2005). Assim, foram identificadas algumas condições que dificultam a propagação do fenômeno tais como: o evelhecimento (Guedes et al., 1996), a estimulação ambiental (Santos-Monteiro et al., 2000), o uso de antioxidantes (El-Bachá et al., 1998; Bezerra et al., 2005), o hipotiroidismo (Guedes e Pereira da Silva, 1993), a hiperglicemia (Costa-Cruz e Guedes, 2001; Costa-Cruz et al, 2006), ativação farmacológica do sistema serotoninérgico, com drogas como a Fluoxetina (Amâncio-dos-Santos et al., 2006) e o Citalopram (Guedes et al, 2002), o tratamento com o antagonista opióide Naloxone (Guedes et al., 1987) e com o agonista colinérgico

muscarínico pilocarpina (Guedes e Cavalheiro, 1997; Vasconcelos et al., 2004; Costa-Cruz et al, 2006; Guedes e Vasconcelos, 2008). Por outro lado, outros experimentos identificaram condições que facilitam a propagação da DAC, tais como: desnutrição no início da vida (Rocha-de-Melo e Guedes, 1997), privação do sono paradoxal (Amorim et al., 1988), consumo de etanol (Guedes e Frade, 1993), ativação do sistema GABAérgico através do Diazepam (Guedes e Cavalheiro, 1997), hipertiroidismo (Santos, 2000) e hipoglicemia (Costa-Cruz e Guedes, 2001).

A administração diária de 300mg/kg/dia de L-Arginina durante o período do aleitamento facilitou, na idade adulta, a propagação do fenômeno da DAC (Frazão, 2004). A suplementação com histidina (que fornece quantidades equivalentes de nitrogênio, mas nada tem a ver com a síntese do óxido nítrico) não reproduziu o mesmo efeito indicando que o efeito é independente da quantidade de nitrogênio fornecida pela L-Arginina (Frazão, 2004). Além disto, a L-Arginina, quando testada em ratos precocemente desnutridos, provocou efeitos menos intensos sobre a DAC, em comparação com aqueles observados nos bem-nutridos (Frazão, 2004).

Na avaliação histológica do córtex visual, neurônios NADPH-d positivos apresentaram resistência à desnutrição, mas mostraram-se susceptíveis à L-Arginina, aumentando a arborização e as varicosidades dendríticas (Maia et al., 2006). Tais informações podem ter relevância nas relações entre nutrição e função visual humana. Mudanças do estado nutricional podem afetar funções básicas neurais, como o processamento da informação visual e a percepção da sensação correspondente, assim como as execuções de tarefas motoras dependentes da visão, e desta forma podem alterar estruturas do córtex visual (Dowdeswell et al., 1995; Powls et al. 1997).

3. METODOLOGIA

Antes de iniciar os procedimentos metodológicos o projeto de pesquisa foi submetido ao comitê de ética tendo sido aprovado (Anexo A). Ratos Wistar machos (n=107), provenientes da colônia do Departamento de Nutrição da Universidade Federal de Pernambuco (UFPE) foram distribuídos em 2 grupos, de 55 animais nutridos e 52 desnutridos, criados em condições favoráveis (ninhadas com 6 filhotes; grupo N6) ou desfavoráveis de lactação (ninhadas com 12 filhotes; grupo N12). Cada um desses 2 grupos foi subdividido em 5 subgrupos, tratados com L-Arginina em três distintas doses (150, 300 e 450 mg/kg/dia), com o veículo (água destilada; grupo 4), ou sem tratamento ("naive"; grupo 5). Tanto a L-Arginina quanto a água destilada foram administradas diariamente por gavagem, do 7º ao 28º dias de vida, no horário das 12 às 14 horas. A dieta de manutenção da mãe e a dieta dos filhotes depois do desmame foi a dieta comercial denominada "Labina" (da firma Purina do Brasil Ltda.) contendo 23% de proteína. A mãe e seus filhotes tiveram livre acesso a água e alimento.

Os animais foram pesados nos dias 7, 14, 21, 25 e no dia do registro da DAC (30-40 dias de vida; mediana de 33dias). Os filhotes foram desmamados aos 25 dias de vida. No dia do registro foi realizada traqueostomia do animal seguida da trepanação de 3 orifícios, um no córtex frontal e dois no parietal, no seu hemisfério direito. O registro (compreendendo o ECoG e a variação lenta de voltagem da DAC), foi realizado em 2 pontos da região parietal durante 4 horas, em um polígrafo MODELO 7D (Grass Medical Instruments), sob anestesia com uma mistura de 1 g/Kg de uretana + 40 mg/Kg de cloralose (ip). A DAC foi provocada a cada 20-30 minutos por estimulação química na região frontal, sendo utilizado para isto o cloreto de potássio (KCl) a 2% (Costa-Cruz e Guedes, 2001). Durante o registro, a temperatura retal do animal foi mantida em 37°C ± 1°C por meio de um aquecedor elétrico. A velocidade de propagação da DAC foi calculada com base na distância entre os dois eletrodos registradores e no tempo gasto pela DAC para percorrer a distância entre os mesmos. A cada hora de registro foi calculada a velocidade média da propagação do fenômeno.

Ao final do registro, o animal era sacrificado por lesão tronco-bulbar, pela introdução de agulha na cisterna magna, e seu encéfalo era retirado. O peso do encéfalo, úmido e seco, foi determinado ao final dos registros utilizando-se para isto uma balança analítica (modelo Bosh, S-2000, com sensibilidade até 0,1mg).

Os dados dos animais que receberam L-Arginina na dose de 300mg/Kg/dia e os animais controle tratados com água foram comparados aos dados de Frazão.

Todos os resultados foram analisados utilizando-se a ANOVA, seguido, se necessário, pelo teste de Tukey. Foram aceitos como significantes as diferenças em que $p \leq 0,05$.

4. RESULTADOS

Nesta tese, foram investigados, em ratos recém-desmamados, os efeitos do tratamento prévio (no aleitamento) com diferentes doses de L-Arginina, associadas a condições favoráveis e desfavoráveis de lactação, sobre as características do fenômeno da depressão alastrante cortical (DAC). Dois artigos científicos originais foram submetidos a revistas internacionais. Adiante estão apresentados os referidos artigos na versão original que foi enviado para as revistas.

O primeiro artigo deste estudo é intitulado: "**L-Arginine administration during rat brain development facilitates spreading depression propagation: evidence for a dose- and nutrition-dependent effect**". Foi submetido como artigo original à revista **Nutritional Neuroscience.** (Anexo B) Explora a área representada pela interface entre Nutrição, Dieta, Neurofisiologia, Neuropsicofarmacologia e Neurologia, sendo classificada como qualis internacional A pela CAPES, possui o fator de impacto 1,349. O segundo artigo deste estudo é intitulado: **"Early malnutrition, but not age, modulates in the rat the L-Arginine facilitating effect on cortical spreading depression"**. Foi submetido como artigo original à revista **Neuroscience Letters**. (Anexo C) É classificada como qualis internacional A pela CAPES, com fator de impacto 2,092. Divulga artigos na área de Neurofisiologia, Neurofarmacologia e Neurologia.

Title: L-Arginine administration during rat brain development facilitates spreading depression propagation: evidence for a dose- and nutrition-dependent effect

Authors: Maia LMSS[1], Amancio-dos-Santos A[2], Duda-de-Oliveira D[1], Angelim MKC[1], Germano PCP[1], Santos SF[1], Guedes RCA[1[CA]].

Address: Depts. of [1]Nutrition and [2] Physiology, Univ. Federal de Pernambuco, 50670901 - Recife, PE, Brasil.

[CA] Corresponding author. Address: Dept. of Nutrition, Univ. Federal de Pernambuco, 50670901 - Recife, PE, Brasil.

E-mails: (1) rc.guedes@terra.com.br (2) rguedes@ufpe.br

Abstract

L-Arginine (ARG) is the precursor of the nitric oxide (NO) synthesis. NO-mediated signaling seems to be involved in the phenomenon of cortical spreading depression (CSD). Here well-nourished and malnourished rats were treated, by gavage, with 150, 300 or 450 mg/kg/d of L-Arginine from postnatal days 7-28, and CSD propagation was analyzed at 30-40d. Compared to non-treated- ("naïve") and water-treated controls, ARG-treated rats dose-dependently displayed higher CSD-velocities ($P<0.05$). In the malnourished rats only the highest ARG-dose (450mg/kg/d) increased CSD velocities. The mean±sd CSD-velocities (in mm/min) were: for well-nourished rats, 3.77±0.15, 3.78±0.23, 4.03±0.16, 4.36±0.19 and 4.41±0.26, in the naïve-, water-controls, 150, 300 and 450 mg/kg/d ARG-groups, respectively; for the same conditions in the malnourished rats, the velocities were 4.18±0.13, 4.22±0.09, 4.24±0.10, 4.27±0.21 and 4.64±0.22, respectively. Results demonstrate a dose- and nutrition-dependent CSD-facilitation by L-Arginine administered during the brain development. It is suggested that this effect is due to the modulation of nitric oxide synthesis.

Key word: Arginine, Brain development, Cortical spreading depression, Malnutrition, Nitric oxide.

Introduction

Arginine (ARG) is considered as an essential amino acid early in life, and is the precursor of the synthesis of nitric oxide (NO), which has important roles in the brain development and function (Garthwaite et al., 1988). The enzyme nitric oxide synthase (NOS) can catalyze NO synthesis in a reaction between L-Arginine and molecular oxygen, in cells and tissues of mammals (Garthwaite et al., 1988), and exogenous L-Arginine can be utilized by the brain to enhance NO production (Hara et al., 2004).

Some studies strongly suggest that nitric oxide-mediated signaling is involved in the phenomenon of cortical spreading depression (CSD; Fabricius et al., 1995; 2006; Scheckenbach et al., 2006). CSD was described as a reversible and propagated "wave" of depression of spontaneous neuronal activity that slowly spreads across the brain cortical surface in response to electrical, mechanical or chemical stimulation of one point on brain tissue (Leão 1944a). In this slow electrophysiological phenomenon, the total recovery process is completed in 5-10 min. Concomitant with the electrocorticogram depression, a slow negative DC-potential change of the cortical surface appears (Leão, 1947). Changes in several brain parameters during CSD have been reported, and this includes alterations in the diameter of the blood vessels of the pia-mater (Leão, 1944b; Read et al., 1997). CSD is considered as a phenomenon related to brain excitability and, as such, has been causally associated to important human diseases like migraine (Lehmenkühler et al., 1993; Read and Parsons, 2000) brain ischemia (Takano et al., 1996) and epilepsy (Leão, 1944, 1972; Guedes and Cavalheiro, 1997).

The developing brain can easily suffer morphological and electrophysiological changes as a consequence of early malnutrition. Depending on its intensity and duration,

nutritional deficiency early in life can modify histological (Borba et al., 2000) and biochemical (Bonatto et al., 2006) patterns of developmental processes in the brain, influencing its functions (Guedes et al., 1996; Morgane et al., 1978; 1993). In developing countries, a considerable proportion of children suffer from malnutrition-related delay in its physical and neuro-psychic development (Casper, 2004; Liu et al., 2003; Onís et al., 2000). Studies using animal models demonstrate that early nutritional deficiency affects the nervous system more severely than late malnutrition (Borba et al., 2000; Hack et al., 1991; Picanço-Diniz et al., 1998; Rocha-de-Melo et al., 2004). For such studies, the albino rat represents a good animal model, since the pups can easily have their nutritional status deteriorated during the suckling period by increasing the litter size, i.e., augmenting the number of pups to be suckled by one dam. This condition implies that each pup receives an insufficient amount of milk, resulting in nutritional deficiency (Costa-Cruz and Guedes, 2001; De Luca et al., 1977; Fernández et al., 1993; Maia et al., 2006; Rocha-de-Melo et al., 2004; 2006).

It has been demonstrated in rats that CSD can be influenced by early malnutrition (De Luca et al., 1977; Rocha-De-Melo and Guedes, 1997; Rocha-De-Melo et al., 2006). Also, preliminary (unpublished) data from our laboratory indicated that administration of an appreciable amount (300 mg/kg/d) of L-arginine during the suckling period to well-nourished, developing rat pups facilitates CSD propagation. In the present study well-nourished and malnourished (large litters technique) rat pups were treated during the lactation period with three distinct doses of L-Arginine in order to evaluate possible changes on CSD propagation, assessed in the post-weaning period. It was hypothesized that (1) exogenous L-Arginine administered during the brain development influences CSD

propagation, (2) this effect is dependent on the L-Arginine dose and (3) the brain nutritional condition modifies the L-Arginine effect on CSD.

Methods

All experiments were carried out in accordance with the "Principles of Laboratory Animal Care" (National Institutes of Health, USA) and were approved by the Ethics Committee for Animal Research of the Universidade Federal de Pernambuco, Brazil. Male Wistar rats (n=107), from the colony of the Department of Nutrition of our university, were suckled under favorable conditions (in litters formed by 6 pups; well-nourished group, n=55) or in unfavorable conditions (12 pups per litter; malnourished group, n=52). The dams were fed a rodent laboratory chow diet (Purina do Brazil Ltd.) with 23% protein. Each nutritional pup-group originated 5 sub-groups, treated, by gavage, respectively with 150, 300 and 450 mg/kg/day L-Arginine (Sigma; experimental groups A150, A300 and A450, respectively), or distilled water (Dw group), or non-treated (naïve – Nv- group). The gavage procedure was carried out daily, between 12 and 14 h, from the postnatal day 7 to 28. After weaning (25 days of life), the pups had free access to water and the mother's diet. Animals were maintained in 51 x 35.5 x 18.5 cm polypropylene cages, in a room with a light-dark cycle (12/12 h; lights on at 6 am) and temperature of 23 ± 1°C. The animals were weighed on days 7, 14, 21, 25 and on the day of the CSD recording (30-40 days of life). For the CSD electrophysiological experiment, the animal was anesthetized with a mixture of 1 g/kg urethane plus 40 mg/kg chloralose intraperitoneally. A tracheal cannula was inserted and three trephine holes were made on the right side of the skull. These holes were aligned in the anteroposterior direction and parallel to the midline (see insert in Figures 3 and 4). One hole was positioned on the frontal bone (2mmin diameter) and was used to apply the

stimulus (KCl) to elicit CSD. The other two holes were drilled on the parietal bone (3–4mm in diameter) and were used to record the propagating CSD wave. Rectal temperature was continuously monitored and maintained at 37±1^{o} C by means of a heating blanket. CSD was elicited at 20 min intervals by 1-min application of a cotton ball (1–2 mm in diameter) soaked with 2% KCl solution (approximately 270 mM) to the anterior hole drilled at the frontal region. Both the slow potential change and the reduction of the spontaneous cortical electrical activity (ECoG) accompanying CSD were recorded for 4 h, by using two Ag-AgCl agar-Ringer electrodes (one in each hole) against a common reference electrode of the same type, placed on the nasal bones. The CSD velocity of propagation was calculated from the time required for a CSD wave to pass the distance between the two cortical electrodes. At the end of the recording session, the animals, while still anesthetized, were killed by lesioning the bulbar region with a sharp needle, inserted through the *cisterna magna*, promptly provoking cardio-respiratory arrest. The brain (including the cerebellum and excluding the olfactory bulb) was immediately removed and weighed (wet weight). It was then kept at 100^{o}C and weighed daily until it reached a constant weight (dry weight). Body and brain weight-, as well as CSD-velocity intergroup differences, were compared by using ANOVA followed by a post hoc (Tukey) test, where indicated. Differences were considered significant when $p \leq 0.05$.

Results

Body weight

Fig. 1 presents the body weight at the different time-points, as described in methods. Malnourished animals, suckled in litters with 12 pups, always displayed lower body weights than the controls, maintained in litters with 6 pups. On the day of the CSD recording session, well-nourished and malnourished animals presented mean body weights ranging from 100.8±11.9g to 115.8±10.5g (well-nourished) and from 72.3±11.9g to 82.5±12.0g (malnourished). No weight differences associated to the L-Arginine administration were seen, as compared with the corresponding Dw- and Nv-controls.

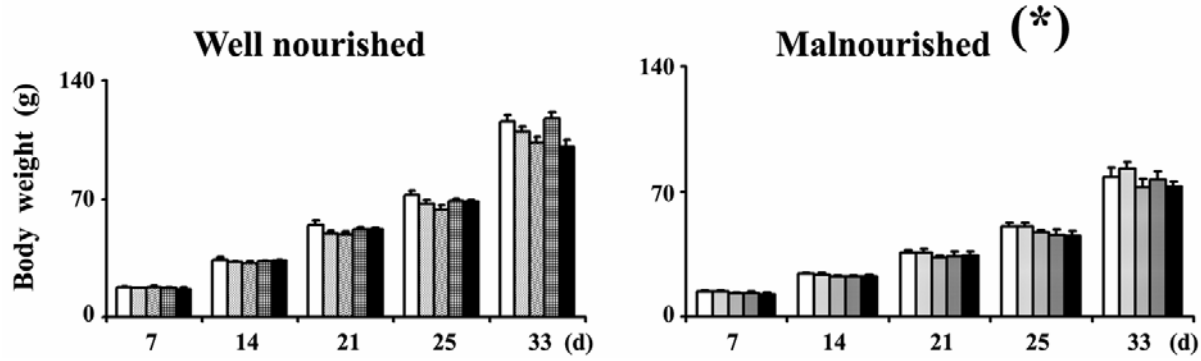

Figure 1: Body weight (mean±standard error of the mean) of well-nourished (litters formed by 6 pups) and malnourished (litters formed by 12 pups) rats, receiving, per gavage, from the 7th to the 28th postnatal days, 150, 300 and 450 mg/kg/d of L-Arginine (three bars at the right side of each bar-cluster), or distilled water (second bar from left), or gavage-free (naïve group; bar at far left). Weights were measured on days 7, 14, 21, 25 and 33 (n ranges from 7 to 11 in each group) . The asterisk indicates that all malnourished values are significantly different from the corresponding well-nourished controls ($p \leq 0.05$; ANOVA plus Tukey test).

Brain weight

Fig. 2 presents the wet- and dry brain weights, measured on the day of the CSD recording. Malnourished animals suckled in litters of 12 pups displayed lower brain weights than the respective controls maintained in litters of 6 pups. The mean wet-brain

weights in well-nourished and malnourished animals ranged from 1.462±0.037g to 1.554±0.055g (well-nourished) and from 1.305±0.097g to 1.434±0.050g (malnourished). The mean dry-brain weights in well-nourished and malnourished animals ranged from 0.287±0.009g to 0.310±0.014g (well-nourished) and from 0.235±0.038g to 0.271±0.022g (malnourished). Among the malnourished rats, the groups treated with 150- and 300 mg/kg/d L-Arginine displayed respectively lower dry- and wet brain weights, as compared with the malnourished Nv-controls.

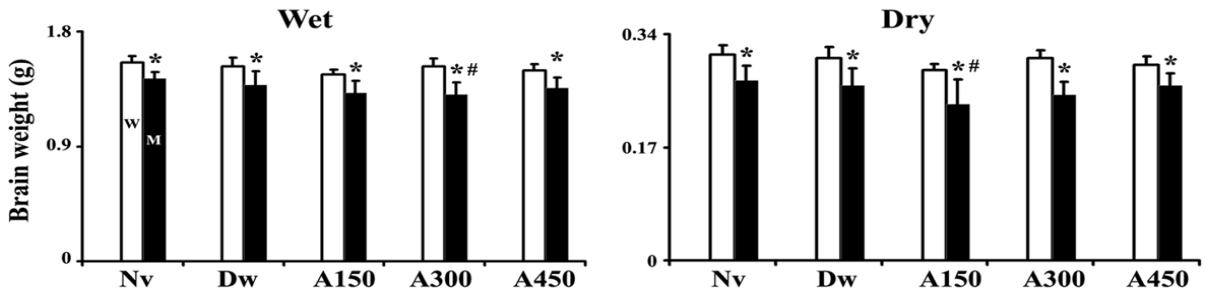

Figure 2: Weights of the wet- (left panel) and dry-brain (right panel) of 30-40 days-old rats receiving, per gavage, from the 7th to the 28th postnatal days, 150, 300 and 450 mg/kg/d of L-Arginine (groups denominated A150, A300 and A450, respectively). They were compared with two control groups: one, treated per gavage with distilled water (Dw group) and the other, not submitted to any gavage-treatment ("naïve" group; Nv). Data are expressed as mean±standard error of the mean. The asterisks indicate that all malnourished values are significantly lower than the corresponding well-nourished ones. The #-symbol denotes L-Arginine group values that are different from the respective Dw-group ones ($p \leq 0.05$; ANOVA plus Tukey test).

CSD propagation

In all groups, topical application of 2% KCl for 1min at the frontal cortex reproducibly elicited a single CSD wave, which was recorded by the two electrodes located more posterior in the stimulated hemisphere. Figs. 3 and 4 are electrophysiological

recordings showing the ECoG depression and the slow potential change accompanying CSD, on the cortical surface of four well-nourished (Fig. 3) and four malnourished (Fig. 4) animals. Both the ECoG and slow potential recordings confirmed the presence of CSD after each KCl-stimulation.

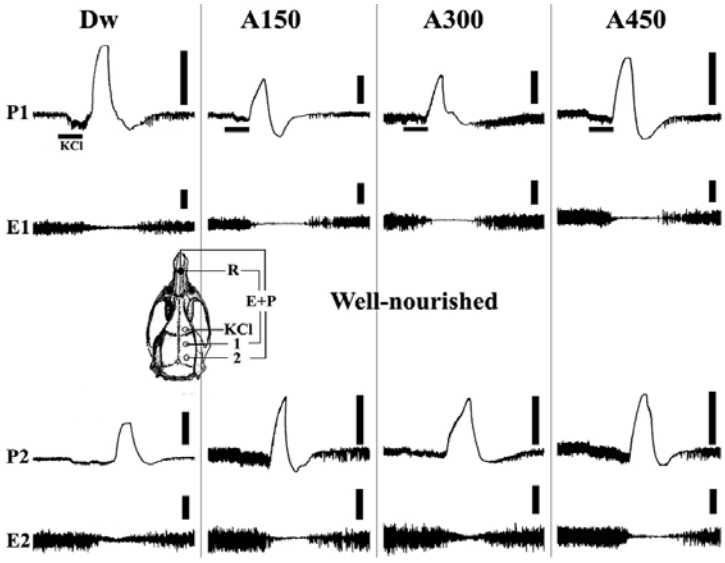

Figure 3: Electrocorticogram (E) and slow potential change (P) recorded during cortical spreading depression (CSD), in 30-40 days-old well-nourished rats, which received, from the 7th to the 28th postnatal days, 150, 300 and 450 mg/kg/d of L-Arginine (groups denominated A150, A300 and A450, respectively, or distilled water (Dw-group). The horizontal bars show the period (1 min) of stimulation with 2% KCl, necessary to elicit CSD. The vertical bars equal -10mV and -1mV, respectively for the P- and E recordings (negativity is upwards). The place of KCl application and of the reference electrode are indicated in the inset, which also shows the recording points 1 and 2 (from which the traces marked at left with the same numbers were recorded). The interelectrode distance is 4.0 mm in all cases.

In the well-nourished rats, the mean±sd CSD-velocities (in mm/min) were 3.78±0.23 and 3.77±0.15 for the Dw- and Nv-groups, respectively, and 4.03±0.16, 4.36±0.19 and 4.40±0.26 for the 150, 300 and 450 mg/kg/d L-Arginine-treated groups. In the malnourished rats, the mean±sd CSD-velocities (in mm/min) were 4.22±0.09 and 4.18±0.13 for the Dw- and Nv-groups, respectively, and 4.24±0.10, 4.27±0.21 and

4.64±0.22 for the 150, 300 and 450 mg/kg/d L-Arginine-treated groups. The mean±sd CSD velocities (in mm/min) for all groups are presented in fig 5.

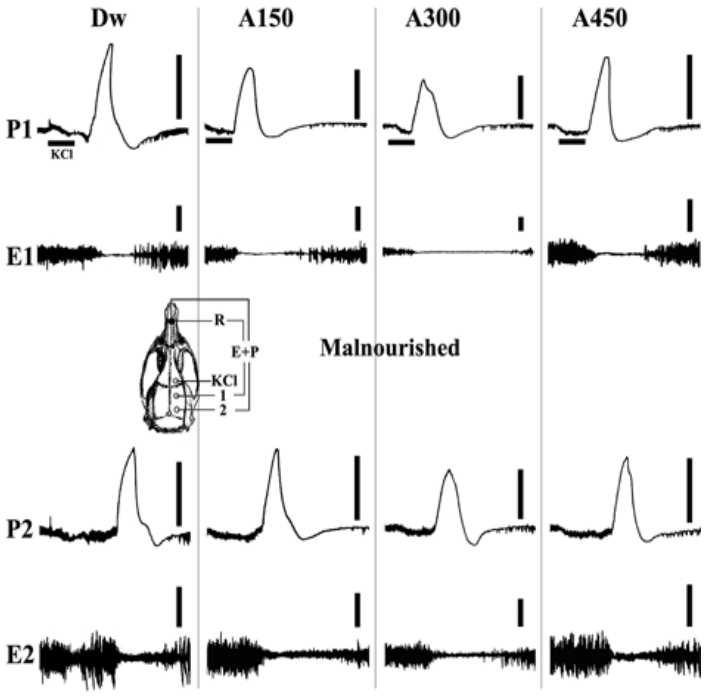

Figure 4: Electrocorticogram (E) and slow potential change (P) recorded during cortical spreading depression (CSD), in 30-40 days-old malnourished rats, which received, from the 7th to the 28th postnatal days, 150, 300 and 450 mg/kg/d of L-Arginine (groups denominated A150, A300 and A450, respectively, or distilled water (Dw-group). The horizontal bars show the period (1 min) of stimulation with 2% KCl, necessary to elicit CSD. The vertical bars equal -10mV and -1mV, respectively for the P- and E recordings (negativity is upwards). The place of KCl application and of the reference electrode are indicated in the inset, which also shows the recording points 1 and 2 (from which the traces marked at left with the same numbers were recorded). The interelectrode distance is 4.0 mm in all cases.

By comparing the three L-Arginine doses used in this study, a dose-dependent effect could be demonstrated, and this effect was modified by the nutritional status of the animals. In the well-nourished condition, the three L-Arginine-treated groups displayed significantly higher CSD velocities ($P<0.05$), as compared to the corresponding control groups (Dw- and Nv-), and the group which received the lowest dose (150 mg/kg/d) of L-Arginine significantly differed from the other two L-Arginine-groups, which presented similarly

higher CSD-velocities (Figure 5, white bars). In the malnourished condition, however, only in the group receiving the highest dose (450mg/kg/d) were the CSD velocities significantly higher than the Nv and Dw groups (Figure 5, black bars).

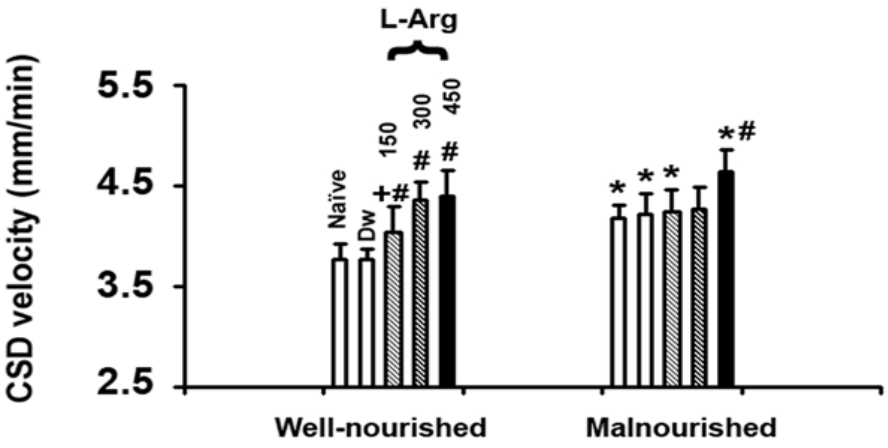

Figure 5: Velocity of propagation of cortical spreading depression (CSD) (mean±standard deviation) of well-nourished (litters formed by 6 pups) and malnourished (litters formed by 12 pups) 30-40 days-old rats, which received, per gavage, from the 7th to the 28th postnatal days, 150, 300 and 450 mg/kg/d of L-Arginine, or distilled water (Dw-control). Another control group did not receive any gavage (naïve group). Asterisks indicate the malnourished groups that are significantly different ($P<0.05$) from their corresponding well-nourished groups. The # symbol indicates the L-Arginine groups that are different from their corresponding Dw groups. The + symbol indicate that the CSD-velocity of the well-nourished group treated with 150mg/kg/d L-Arginine is, different from the other two L-Arginine treated well-nourished groups (ANOVA plus Tukey test).

Discussion

The main finding of the present study was that the daily administration of L-Arginine during the period of fast brain development facilitated CSD propagation, as evaluated by the increases in its propagation velocity. As a rule, this CSD effect was more intense in the groups treated with the higher doses of L-Arginine, suggesting a dose-response relationship. Furthermore, in the malnourished rats the L-Arginine effect on CSD was significant only at the highest dose (450 mg/kg/d), indicating that early-in-life malnutrition attenuated the L-Arginine effect. The data support the hypothesis of an L-Arginine-mediated facilitating process in CSD and raise the question of how such an amino acid intake might increase the CSD susceptibility in the developing brain. The most reasonable possibility is that this effect is mediated by the increased nitric oxide synthesis, as a consequence of the treatment with exogenous L-Arginine (Hara et al., 2004), which alters the vascular reactivity, i.e., the blood flow, in the cerebral blood vessels.

CSD-associated changes in the brain circulation are known since the first descriptions of the CSD features (Leão, 1944b), and this association has been later confirmed, reinforcing the idea of a relationship between CSD and migraine (Lauritzen, 1987; 2001). Experimental evidence in rats indicates the participation of the Arginine-Nitric oxide pathway in the cerebrovascular reactivity during CSD (Fabricius et al., 1995; Scheckenbach et al., 2006). Based on this evidence, it is reasonable to predict that the treatment with three different doses of exogenous L-Arginine during 21 d, as in the present study, probably changed the brain nitric oxide synthesis, influencing the CSD susceptibility. As discussed in a previous study (Maia et al., 2006), the here described CSD changes, assumed to be causally associated to the L-Arginine treatment, cannot be

attributed to the animal gavage stress since the control group has been submitted to the same gavage procedure (with distilled water) and did not present those CSD alterations. In addition, the gavage-free control group ("naïve" rats) displayed CSD-results similar to the distilled water controls. It must also be reinforced that the CSD-effects varied as a function of the L-Arginine dose. This is in line with the conclusion that L-Arginine administration actually was the responsible for the dose-dependent CSD propagation increase.

The doses of L-Arginine presently administered were within the dose-range usually employed to test some L-Arginine-effects on the central nervous system. According to previous reports, such doses vary from 37.5mg/kg to 600 mg/kg (Cherian et al., 2003; Smriga and Torii, 2003; Hara et al., 2004). In animal experiments, L-Arginine in that dose-range is reported to protect the brain against the anxiety induced by stress (Smiriga and Torii, 2003). A protective action of L-Arginine against the effects of impact brain injury has been described to be dose-dependent (Cherian et al., 2003). So, the present study, which reports dose-related effects of L-Arginine on CSD propagation, can be considered as novel electrophysiological evidence in favor of dietary amino acids effects on the brain.

Exogenous L-Arginine can change brain excitability, probably by influencing nitric oxide synthesis (Kim et al., 2004; Ma et al., 1995) and this is clinically important, concerning excitability-related diseases, like epilepsy (Capasso et al, 2003). In laboratory animals, under certain conditions a close relationship between changes in brain excitability and in CSD has also been reported (Guedes and Cavalheiro, 1997; Guedes and De Vasconcelos, 2008; Koroleva et al., 1993). Considering the above-mentioned possibility of brain excitability changes due to L-Arginine administration, it can be concluded that the present results represent one of those situations in which L-Arginine-nitric oxide

relationships influence brain electrical activity (Murashima et al., 2000; Prast and Philippu, 2001).

Altered intakes of different amino acids can lead to changes in metabolic responses (Young and Marchini, 1990). The nutrition- and metabolism status of the lactating dam can influence the development of the suckling organism (Koletzko et al., 1998). In this work, suckling rat pups have been treated daily with additional doses of exogenous L-Arginine, and the susceptibility of the cerebral cortex to CSD has been evaluated by changes in its propagation velocity. To our knowledge, this constitutes the first report on that specific subject. Although amino acid blood levels have not been monitored, it is reasonable to assume that this treatment, carried out during 21 days, might in all probability have caused an amino acid imbalance (Jessop, 1997), due to the increase of arginine blood levels (Gietzen, 1993; Gietzen et al., 1998).

The reduced body and brain weights of the malnourished animals, in comparison to the well-nourished ones, indicate that increasing the litter size was effective in impairing the nutritional status of the pups during the suckling period, impairing the organism development, with repercussions on the weights of vital organs, such as the brain. This might increase the probability of altering their functions (Morgane et al. 1978, 1993; Cintra et al., 1997). The present data on body- and brain weight reduction are similar to those of previous studies using the same litter-size manipulation to alter the nutritional condition of the sucklings (De Luca et al., 1977; Fernández et al., 1993; Maia et al., 2006; Rocha-de-Melo et al., 2004; Tonkiss et al., 1988).

The malnourished rats not treated with L-Arginine (naïve and water-treated groups) presented higher CSD velocities as compared with the corresponding well-nourished groups (Figure 5). It is tempting to postulate that the imbalance in the amount of dam's

milk available for each malnourished pup might play an important role in producing the CSD-propagation changes presently demonstrated. The complete understanding of the underlying mechanisms responsible for the effects of early malnutrition on CSD propagation still requires further investigation. The participation, on the CSD-effects, of factors like nutrition-dependent changes in brain gliogenesis, myelination and transmitter activity, as well as in the brain cell packing density, has been postulated by several investigators, based on evidence obtained in the early-malnourished rat (De Luca et al. 1977; Guedes et al. 1987, 1992; Rocha-de-Melo and Guedes 1997). All the above factors have been shown to be determinant in establishing the brain susceptibility to CSD (De Luca et al. 1977; Rocha-de-Melo et al., 2006; Amâncio-dos-Santos et al., 2006).

In the malnourished rat brain, the CSD-effects of some drugs are similar to those found in the well-nourished animals (Amâncio-dos-Santos et al., 2006), whereas other compounds like glucose (Ximenes-da-Silva and Guedes, 1991), and diazepam (Guedes et al., 1992) are diminished, when compared to their effects on well-nourished animals. In the malnourished rats of the present study the L-Argine effect on CSD propagation seemed to be diminished, as only the highest dose produced significant CSD velocity increases. Taken together, the evidence collectively indicates reduction of the effectiveness of pharmacological compounds that is associated to malnutrition during brain development. If this assumption could be proven to occur in the human species, then the possibility that a determined anti-migraine, or a anti-epileptic drug could have different degrees of effectiveness, depending on the previous nutritional status, would have to be considered.

In conclusion, this study documents, for the first time, the facilitating effects of L-Arginine on CSD, analyzing in detail the two following novel observations: first, the application, by gavage, of L-Arginine during the fast brain development period increased

the propagation of CSD in the rat cortical surface; second, this effect increased with the L-Arginine dose; third, malnutrition early in life reduced it. The suggestion that this effect is due to the modulation of nitric oxide synthesis by the L-Arginine treatment is a tempting hypothesis, which must be further investigated.

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Title: Early malnutrition, but not age, modulates in the rat the L-Arginine facilitating effect on cortical spreading depression

Authors: Marília Ferreira Frazão, Luciana Maria Silva de Seixas Maia and Rubem Carlos Araújo Guedes*.

Address: Dept. of Nutrition, Univ. Federal de Pernambuco, 50670901 Recife, Brasil.

* Corresponding author: address as above.

e-mail: (1) rc.guedes@terra.com.br (2) guedes.rca@gmail.com

Abstract

Nutritional factors acting during brain development can permanently alter brain electrophysiology. L-Arginine is the precursor of nitric oxide synthesis, which can modulate brain function. Here we investigated the effect of early-in-life administration (during postnatal days 7-28) of L-Arginine (300 mg/kg/d) on cortical spreading depression (CSD), recorded in well-nourished and malnourished (large litters technique) rats aged 30-40d (young) and 90-110d (adult). Compared to water-treated controls, well-nourished L-Arginine-treated rats, but not the malnourished ones, displayed higher CSD-velocities ($P<0.05$) at both ages. The mean±sd CSD-velocities (in mm/min) were: for water- and L-Arginine well-nourished rats, 3.78±0.23 and 4.36±0.19 (young groups), and 3.28±0.16 and 4.09±0.30 (adult); for the same conditions in the malnourished rats, 4.22±0.09 and 4.27±0.21 (young), and 4.11±0.18 and 4.21±0.33 (adult). L-Arginine treatment did not affect body- and brain weights. It is concluded that early L-Arginine treatment long lastingly increased brain CSD-susceptibility and this effect is abolished by early malnutrition.

Key words: Age, Arginine, Brain development, Cortical spreading depression, Malnutrition, Nitric oxide.

As an essential precursor for the synthesis of protein molecules with enormous biological importance, L-Arginine displays remarkable metabolic and regulatory versatility [3,37]. Nitric oxide (NO), generated from arginine, has multifunctional roles in the nervous system, including modulation of excitability and neurotransmitter release [29,39,53], regulation of local cerebral blood flow, neuroprotection and synaptic plasticity [4,10,33,40,43]. In cells and tissues of mammals, NO is synthesized in a reaction involving L-Arginine and molecular oxygen, which is catalyzed by the enzyme nitric oxide synthase [12], and exogenous L-Arginine can be utilized by the brain to enhance NO production [17].

Several studies suggest that NO-mediated signaling is involved in the phenomenon of cortical spreading depression (CSD) [18,45,50,54]. CSD is an interesting excitability-related neural phenomenon, which has been first described as a cortical response consequent to electrical, mechanical or chemical stimulation of the tissue surface [23]. This response consists of a reversible and slowly propagating “wave” of reduction of the spontaneous and evoked cortical electrical activity, with a simultaneous DC slow potential change of the tissue [25]. CSD has been studied in several animal species [13], having also been recorded in the human brain [31]. As a phenomenon related to brain excitability, CSD has been causally associated to important human diseases like migraine [27,44] brain ischemia [51] and epilepsy [23,26,15].

Early malnutrition can change patterns of developmental processes in the brain, and this can alter neural functions [34,35]. It is known that nutritional deficiency early in life affects the nervous system more severely than late malnutrition, and some of the effects can

became permanent [5,16,49]. Reports on malnutrition-associated lasting nervous system alterations also includes emotion-, motivation- and anxiety disorders [28], as well as morphological [5,30], physiological, behavioral and biochemical disturbances [46]. For experimental studies on early malnutrition, the albino rat is a good animal of choice, because it is relatively easy to impair the pups' nutritional status by increasing the litter size, i.e., augmenting the number of pups to be suckled by one dam. Under such condition, each pup receives an insufficient amount of milk, resulting in nutritional deficiency [7,8,11,30,47,49]. This malnutrition technique has been used in the present study in order to investigate the effects of L-Arginine treatment in adult rats previously malnourished during the period of fast brain development. By using electrophysiological recording of CSD, two questions in the young and adult brain subjected to perinatal malnutrition followed by nutritional recovery have been presently addressed: (1)How does daily administration of exogenous L-Arginine during the brain development affects CSD propagation, and (2) if so, how would this effect be influenced by the early brain nutritional condition, as well as by age.

Experiments were carried out in accordance with the "Principles of Laboratory Animal Care" (National Institutes of Health, Bethesda, USA) and were approved by the Ethics Committee for Animal Research of the Universidade Federal de Pernambuco, Brazil. Male Wistar rats (n=121) from the colony of our University were used. They were suckled under favorable conditions (in litters formed by 6 pups; well-nourished group, n=63) or in unfavorable conditions (12 pups per litter; malnourished group, n=58). The dams were fed

a rodent laboratory chow diet (Purina do Brazil Ltd.) with 23% protein. Each nutritional pup-group originated 2 sub-groups, treated, by gavage, respectively with distilled water (Dw group; 23 young- and 40 adult rats), or 300 mg/kg/day L-Arginine (Sigma; experimental group A300; 22 young- and 36 adult rats). The gavage procedure was carried out from the postnatal day 7 to 28, between 12 and 14 h. Animals were housed in 51 x 35.5 x 18.5 cm polypropylene cages, in a room with a light-dark cycle (12/12 h; lights on at 6 am) and temperature of 23 ± 1°C. After weaning (25 days of life), the pups had free access to water and to the mother's diet. CSD recording was carried out when the pups were 30-40 days old (n=45; young groups), or when they were 90-110 days old (n=76; adult groups). On the day of the CSD electrophysiological experiment, the animal was intraperitoneally anesthetized with a mixture of 1 g/kg urethane plus 40 mg/kg chloralose. A tracheal cannula was inserted and three trephine holes, aligned in the anteroposterior direction and parallel to the midline, were made on the right side of the skull (see insert in Figure 1). One hole was positioned on the frontal bone (2mm in diameter) and was used to apply the stimulus (KCl) to elicit CSD. The other two holes were drilled on the parietal bone (3–4mm in diameter) and were used to record the propagating CSD wave. Rectal temperature was continuously monitored and maintained at 37±1^{o} C by means of a heating blanket. CSD was elicited at 20 min intervals by 1-min application of a cotton ball (1–2 mm in diameter) soaked with 2% KCl solution (approximately 270 mM) to the anterior hole drilled at the frontal region. CSD electrophysiological features (reduction of the ECoG amplitude and appearance of the slow potential change) were recorded for 4 h, by using two Ag-AgCl agar-Ringer electrodes (one in each hole) against a common reference electrode of the same type, placed on the nasal bones. Calculation of the CSD velocity of propagation was based on the time required for a CSD wave to pass the distance between the two cortical

electrodes. At the end of the recording session, the animals, while still anesthetized, were killed by lesioning the bulbar region with a sharp needle, inserted through the *cisterna magna*, promptly provoking cardio-respiratory arrest. The brain (including the cerebellum and excluding the olfactory bulb) was immediately removed and weighed. Body and brain weight-, as well as CSD-velocity intergroup differences, were compared by using ANOVA followed by a post hoc (Tukey) test, where indicated. Differences were considered significant when $p \leq 0.05$.

As shown in table 1, early-malnourished rats presented lower body- and brain weights at 30 days of age, as compared to the well-nourished ones. When the pups attained the adult age (90 days), no body weight difference was seen, that could be causally related to early malnutrition. However, the brain weight of the malnourished animals remained lower than those of the well-nourished rats at 90 days. L-Arginine treatment did not change the body- and brain weights, as compared to the corresponding controls, treated with distilled water.

Table 1 – Body- and brain weights (mean±sd), evaluated at 30-40d (young rats) and at 90-110d (adult rats). W and M are well-nourished and malnourished rats. ARG and DW are groups treated per gavage, during postnatal days 7 to 28, with 300mg/kg/d L-Arginine and distilled water, respectively. The number of animals in each group is shown in parentheses. Values with superscript letters are different ($P<0.05$) from the corresponding values of the group marked with the same letters at the left column (ANOVA plus Tukey test).

GROUP	**Body weight (g)**	**Brain weight (g)**
Young rats (30-40 days old)		
[a] DW-W (10)	109.4 ± 9.3	1.523 ± 0.073
[b] ARG-W (11)	111.4 ± 18.6	1.529 ± 0.060
[c] DW-M (9)	82.5 ± 12.1[a]	1.385 ± 0.105[a]
[d] ARG-M (8)	76.5 ± 13.3 [b]	1.305 ± 0.097[b]
Adult rats (90-110 days old)		
[e] DW-W (10)	348.6 ± 38.8	1.6915 ± 0.048
[f] ARG-W (11)	367.6 ± 40.7	1.6606 ± 0.072
[g] DW-M (9)	330.2 ± 25.2	1.5108 ± 0.041[e]
[h] ARG-M (8)	341.0 ± 30.3	1.5247 ± 0.126[f]

Figure 1 shows representative electrophysiological recordings, documenting the ECoG depression and the slow potential change accompanying CSD in two well-nourished and two malnourished 30-40 days old rats, treated with distilled water and L-Arginine (one well-nourished and one malnourished animal on each treatment). Recordings at 90-110 days of life (not shown) displayed similar electrographic features. As a rule, a single CSD wave was elicited after each application of 2% KCl for 1min at a point of the frontal cortex. After being elicited, this CSD-wave was recorded by the two electrodes located more posterior in the stimulated hemisphere. Both the ECoG and slow potential recordings confirmed the presence of CSD after each KCl-stimulation.

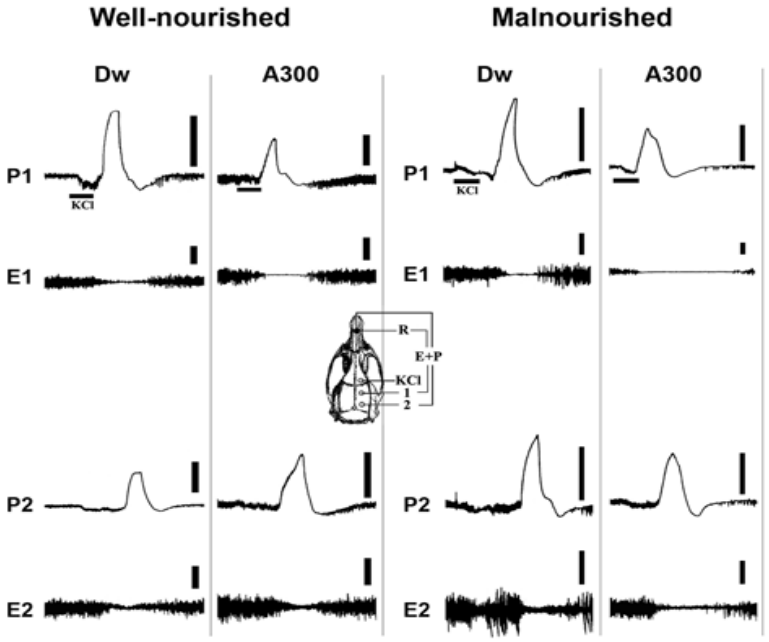

Figure 1 – Electrocorticogram (E) and slow potential change (P) recorded during cortical spreading depression (CSD), in two well-nourished and two early-malnourished 30-40 days-old rats, which received, per gavage, from the 7th to the 28th postnatal days, distilled water (Dw groups), or 300 mg/kg/d of L-Arginine (A300 groups). The horizontal bars show the period (1 min) of stimulation with 2% KCl, necessary to elicit CSD. The vertical bars equal -10mV and -1mV, respectively for the P- and E recordings (negativity is upwards). The places of KCl application and of the reference electrode are indicated in the inset, which also shows the recording points 1 and 2 (from which the traces marked at left with the same numbers were recorded). The interelectrode distance is 4.0 mm in all cases.

In the well-nourished L-Arginine-treated rats, CSD velocities of propagation were higher, as compared with the water-treated controls, both in the young and in the adult ages. In the malnourished groups, no CSD propagation differences associated to the L-Arginine treatment were seen. The mean±sd CSD-velocities (in mm/min) in the well-nourished rats were 3.78±0.23 and 4.36±0.19 for the 30-40 days old Dw- and L-Arginine groups. At adulthood (90-110 days), the mean velocities were 3.28±0.16 and 4.09±0.30 mm/min. In the malnourished condition, the mean±sd CSD-velocities were 4.22±0.09 and 4.27±0.21 for the young (30-40 days old) Dw- and L-Arginine groups, whereas at adulthood (90-110d) the CSD velocities were 4.11±0.18 and 4.21±0.33 mm/min (Figure 2).

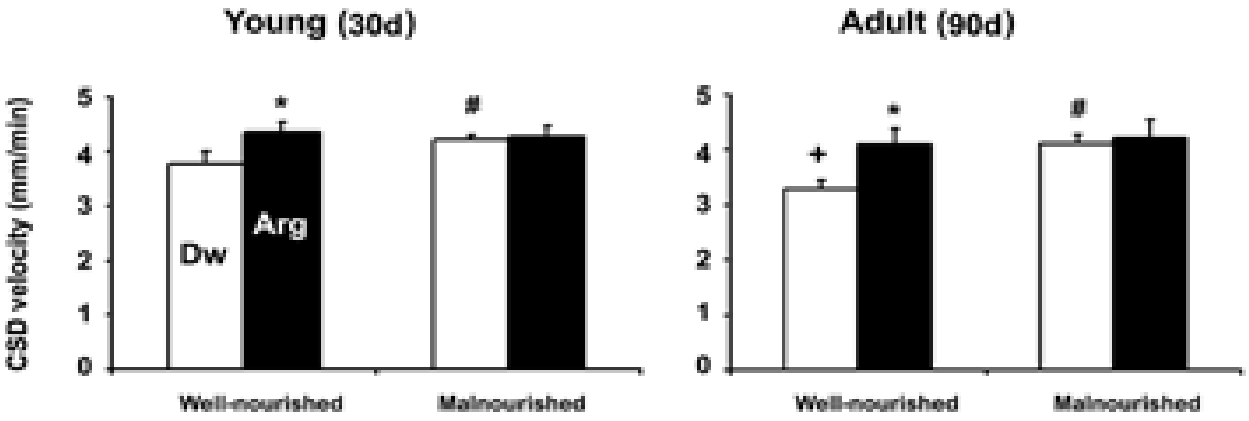

Figure 2 – Mean (±standard deviation) velocity of propagation of cortical spreading depression (CSD) of well-nourished (litters formed by 6 pups) and malnourished (litters formed by 12 pups) young (30-40 days-old) and adult (90-110 days old) rats, which received distilled water (Dw) or 300 mg/kg/d of L-Arginine, per gavage, from the 7th to the 28th postnatal days. Asterisks indicate the L-Arginine treated groups that displayed CSD velocities significantly higher than the corresponding Dw-controls. The # symbols indicate the malnourished groups that present CSD velocities higher than the corresponding well-nourished groups. The + symbol denotes the well-nourished adult group that propagated CSD at a lower velocity, in comparison with the corresponding well-nourished young group (ANOVA plus Tukey test).

In the present study we demonstrated that the administration of 300mg/kg/d of L-Arginine to developing rats was effective in facilitating CSD, as indicated by the higher propagation velocities, in comparison to the water-treated controls. In the two age-ranges that we investigated –young and adult-, the facilitating effect of L-Arginine was the same, suggesting that this effect is long lasting and is not influenced by the age of the animal. Furthermore, in the malnourished rats the L-Arginine did not change the CSD velocity, indicating that malnutrition is a factor that modulates the L-Arginine action on CSD. These results collectively point to a facilitating process in CSD propagation, which is probably mediated by L-Arginine, and is modulated by the early nutritional conditions of the developing brain. One tempting hypothesis to explain our results is based on the mediation by the nitric oxide synthesis, which would be increased as a consequence of the daily treatment with 300mg/kg of L-Arginine [17]. This treatment might in all probability alter the blood vessels reactivity [2], and this might change the cerebral blood flow.

During CSD the cerebral circulation changes [24]. This has called the attention of several researchers, who have dedicated a considerable amount of their time to investigate the possible relevance of CSD-associated changes in the brain circulation in contributing to the mechanisms of certain neurological diseases in which vascular alterations are present. This is the case, as referred above (see introduction), of migraine [21, 22] and brain ischemia [51]. The involvement of the nitric oxide in CSD has been supported by several pieces of experimental evidence [9, 42, 50, 54] which in conjunction with the present results led us to postulate that the administration of exogenous L-Arginine during 21 days, is very likely to change the brain nitric oxide synthesis [2], and this would influence the

CSD features [42]. The confirmation of this possibility will require the future measurement of changes of the brain nitric oxide levels, as a result of the L-Arginine treatment.

Administration of L-Arginine alters brain excitability [53] and this effect is probably mediated by L-Arginine-dependent changes in nitric oxide synthesis [19,29,39]. Changes in brain excitability have also been demonstrated to influence CSD [14,15,20]. Taken together, data suggest that the present L-Arginine treatment influenced CSD propagation probably with the involvement of changes in brain electrical activity [36,38,43]. Similar effects of L-Arginine have been reported by others in developing rats under pentylenetetrazol-induced seizures [41].

The fact that no age-related changes in CSD were detected in the cortical tissue of rats aged from 30-40 days to 90-110days is congruent with previous studies showing age-dependent alterations in the L-Arginine-NO pathway in the rat brain [32]. These authors reported L-Arginine-NO pathway changes in homogenates of brain subcortical structures, but not in the cortical homogenates, suggesting that the rat cortex could be refractory, or at least more resistant than subcortical structures to the pharmacological manipulation of the NO system.

Concerning the malnourished groups, the present results indicate that increasing the litter size was effective in impairing the nutritional status of the pups during the brain development period. Impairment of brain development can be inferred from the reduced body and brain weights, as well as from morphological and physiological alterations of the malnourished animals, in comparison to the well-nourished ones, as largely demonstrated [6,8,11,30,34,35,49,52]. The higher CSD velocities observed in the malnourished Dw-group indicate a facilitating effect of early malnutrition on CSD, what confirms previous

reports on this and other malnutrition models [8,48]. Current discussions on the underlying mechanisms responsible for the effects of early malnutrition on CSD propagation frequently include nutrition-dependent changes in processes like brain gliogenesis, myelination and transmitter activity, as well as in the brain cell packing density [8,48]. These processes are important for the establishment of the CSD features in normally developed rats.

A number of substances can modify CSD propagation when injected systemically. This is the case of glucose [55], diazepam [14] and fluoxetine [1]. However, when applied in early-malnourished animals, those substances do not change CSD, which indicates that early malnutrition renders the developing brain less responsive to the CSD effects of the drugs. In this context the present results, on the failure of L-Arginine in changing CSD propagation in the early-malnourished rats, deserve a special comment. The above-mentioned previous results on malnourished rats, as well as the present ones, collectively show a malnutrition-related effectiveness reduction of substances with metabolic or pharmacological action on the brain. The possibility that this effect occur in the human brain force us to consider the plausible situation in which a certain anti-migraine or an anti-epileptic drug would be less effective, if administered to a patient who had been malnourished early in life. Clinical studies shall address this issue in the future.

In conclusion, the two following novel findings have presently been documented: first, treatment with L-Arginine during brain development facilitated the CSD propagation in the rat cerebral cortex; second, this effect is modulated by the early nutritional status, but not by the age of the animal. Data may shed some light on the understanding of L-Arginine

mediated mechanisms involved in brain excitability-related electrophysiological phenomena.

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5. CONSIDERAÇÕES FINAIS

De acordo com o que foi aqui apresentado, pode-se concluir que a administração de L-Arginina durante o período de desenvolvimento rápido do encéfalo é um procedimento capaz de modificar as características de propagação da depressão alastrante cortical. Nesse sentido, a rota da produção do óxido nítrico a partir da L-Arginina pode ter um importante papel no funcionamento neural. Vários experimentos tentam esclarecer o efeito da L-Arginina em várias fases da vida e também compreender melhor seu papel no sistema nervoso central. Nesse contexto, o presente trabalho representa uma contribuição original para se entender as relações entre esse aminoácido e um fenômeno eletrofisiológico cerebral. O trabalho demonstra também que a dose de L-Arginina administrada ao organismo tem um papel crítico na produção das alterações ora descritas.

Apesar de estar sendo bastante estudada no que diz respeito aos processos etiológicos de doenças neurodegenerativas no envelhecimento, a L-Arginina tem sido usada de forma quantitativamente indiscriminada principalmente por atletas, especialmente na adolescência, na busca de melhoria de rendimento físico. Sabe-se que excesso de óxido nítrico pode potencializar a apoptose neuronal, e que os dados experimentais quanto a doses adequadas de Arginina ainda não são conclusivos, tornando assim tão motivante estudar o papel desse aminoácido no sistema nervoso central.

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ANEXO A – Parecer do Comitê de Ética em Pesquisa

Universidade Federal de Pernambuco
Centro de Ciências Biológicas

Av. Prof. Nelson Chaves, s/n
50670-420 / Recife - PE - Brasil
fones: (55 81) 2126 8840 | 2126 8351
fax: (55 81) 2126 8350
www.ccb.ufpe.br

Ofício nº 023/06 Recife, 08de junho de 2006

Da Comissão de Ética em Experimentação Animal (CEEA) da UFPE
Para: Prof. Rubem Carlos Araújo Guedes
Departamento de Nutrição - UFPE

Os membros da Comissão de Ética em Experimentação Animal do Centro de Ciências Biológicas da Universidade Federal de Pernambuco (CEEA-UFPE) avaliaram seu projeto de pesquisa intitulado **"Curva dose-resposta da administração de L-arginina em ratos lactentes normais e desnutridos: efeitos sobre o fenômeno da depressão alastrante cortical".**

Concluímos que os procedimentos descritos para a utilização experimental dos animais encontram-se de acordo com as normas sugeridas pelo Colégio Brasileiro para Experimentação Animal e com as normas internacionais estabelecidas pelo National Institute of Health Guide for Care and Use of Laboratory Animals as quais são adotadas como critérios de avaliação e julgamento pela CEEA-UFPE.

Encontra-se de acordo com as normas vigentes no Brasil, especialmente a Lei 9.605 – art. 32 e Decreto 3.179-art 17, de 21/09/1999, que trata da questão do uso de animais para fins científicos.

Diante do exposto, emitimos **parecer favorável** aos protocolos experimentais realizados.

Atenciosamente,

Prof. Silene Carneiro do Nascimento
Presidente CEEA

ANEXO B – Documentação de encaminhamento do artigo “L-Arginine administration during rat brain development facilitates spreading depression propagation: evidence for a dose- and nutrition-dependent effect” ao periódico Nutritional Neuroscience.

De: Prasad, Chandan Para: rc.guedes Data: 18/08/08 09:56
Assunto: MS#NN 59408/CP

Texto:
August 18, 2008

Prof. R C A Guedes, MD, PhD
Departmento de Nutrição Centro de Ciências da Saúde Univ. Federal de Pernambuco
50670-901 Recife/PE, BRASIL
E-mail: rc.guedes@terra.com.br

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AUTHOR(S): Maia LMSS, Amancio-dos-Santos A, Duda-de-Oliveira D, Angelim MKC, Germano PCP, Santos SF, Guedes RCA

TITLE: L-Arginine administration during rat brain development facilitates spreading depression propagation: evidence for a dose- and nutrition-dependent effect

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Sincerely yours,
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Chandan Prasad, PhD Professor and Chair
Department of Nutrition and Food Sciences
Editor-in-Chief: Nutritional Neuroscience
Associate Editor: Current Topics in Nutraceutical Research
PO Box 425888
Texas Woman's University
Denton, TX 76204-5888
Tel: +1 940-898-2636
FAX: +1 940-898-2634
E-mail: CPrasad@mail.twu.edu

ANEXO C – Documentação de encaminhamento do artigo "Early malnutrition, but not age, modulates in the rat the L-Arginine facilitating effect on cortical spreading depression" ao periódico Neuroscience Letters

De: "Neuroscience Letters" nsl@elsevier.com
Para: rc.guedes@terra.com.br
Cópia:
Data: 17 Aug 2008 03:19:51 +0100
Assunto: Neuroscience Letters Submission Confirmation

Dear Professor Guedes,

Your submission entitled "Early malnutrition, but not age, modulates in the rat the L-Arginine facilitating effect on cortical spreading depression" has been received for consideration in Neuroscience Letters.

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Maia, Luciana Maria Silva de Seixas
L-Arginina facilita a depressão alastrante cortical, de forma dependente da dose, em ratos nutridos e precocemente desnutridos / Luciana Maria Silva de Seixas Maia. – Recife : O Autor, 2008.
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Inclui bibliografia e anexos.

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